IDENTIFICATION AND FIELD ECOLOGY OF A FUNGAL PATHOGEN OF *OLIGONYCHUS PRATENSIS* AND *TETRANYCHUS URTICAE* (ACARI: TETRANYCHIDAE): *NEOZYGITES* SP. (ENTOMOPHORALES: NEOZYGITACEAE)

by

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CHAPTER 1

GENERAL INTRODUCTION AND AREA DESCRIPTION

GENERAL INTRODUCTION

Spider mite damage and management difficulty. The Banks grass mite (BGM), Oligonychus pratensis (Banks), and the twospotted spider mite (TSM), Tetranychus urticae Koch, often cause substantial yield reduction in field crops in the western Great Plains. Radke (1975) and Pickett (1985) summarize the pest status and distribution of BGM and TSM in this area and other areas of the western United States. Brooks et al. (1986) refer to an eastern-Colorado study that reports an average corn grain-yield reduction of 23% due to BGM in 1984 and 1985. Corn yield reductions of 50 to 70% can occur from heavy mite feeding during grain fill (Sloderbeck et al. 1985). Spider mite feeding directly and indirectly reduces ensilage-yield through grain-yield reduction, stalk rot, and premature leaf loss. The accelerated dry-down of spider mite-infested corn sometimes necessitates early harvest or the time-consuming addition of water to restore ensilage to the proper moisture.

Control of spider mites on corn is more difficult than control of other arthropods on corn. A general trend toward miticide resistance is common throughout the region. Several factors causing mite management difficulty, including resistance to miticides, are discussed by Hirnyk (1983) and Sloderbeck et al. (1985). Others have also documented resistance to miticides and several researchers in in the Western High Plains have recently begun a program to establish base LC_{50} levels for contemporary miticides with which to monitor future miticide resistance (L. L. Buschman unpublished data).

Nearly all miticides are aerially-applied in the High Plains. There is evidence that a significant percentage of aerially-applied pesticides do not reach the portion of the corn plant most often infested by spider mites (D. K. Kuhlman unpublished data). Poor plant coverage coupled with resistance to miticides results in disappointing and costly mite control failures. The tremendous reproductive potentials of spider mites (Tan and Ward 1976, Perring 1983, Margolies & Kennedy 1984) and the inability of most miticides to kill mite eggs, frequently necessitate multiple pesticide applications. These factors increase corn production costs, often with little relief from spider mites.

Banks grass mites, TSM, European corn borers, Ostrinia nubilalis (Hubner), and southwestern corn borers, Diatraea grandiosella (Dyar) often occur in the same fields at the same time. Because the population dynamics and biology of each are different, attempts to control one may not control the others, and may accentuate problems with the others (Sloderbeck et al. 1985). This is particularly true of spider mites following corn borer control attempts. Several corn borer insecticides have been reported to decimate natural enemies of spider mites, leading to unchecked increases in mite numbers. To reduce spider mite increase following insecticide applications, corn producers often combine miticides with insecticides (Buschman & Sloderbeck 1983). In addition, TSM tend to develop resistance to miticides more quickly than BGM (Sloderbeck et al. 1985), which means control of TSM is much more difficult.

An important concept in the management of crop pests is the economic threshhold. Economic thresholds are believed to be essential to establishing the need for and timing of insect control or suppression measures (Coppel & Mertins 1977), but just as mite control is complicated, so is development of threshholds. Several factors interfere with the establishment of economic thresholds for spider mites including: 1) A poor understanding of feeding damage; 2) difficulty in duplicating mite damage in research plots (Feese 1976, Buschman 1982); 3) crop variety and planting date interactions; 4) unpredictable and uneven distribution of mite populations; and 5) the effects of predators and climate. Spider mite research requires extensive time and labor expenditures at critical times (such as pre-/post-treatment counts) during experimental plot work. Based on personal experience, even if economic thresholds were developed, those making control recommendations would find it difficult to obtain sufficient data in a reasonable amount of time on which to base control decisions. Difficulty in reaching control decisions can lead to excessive, untimely, or unnecessary miticide applications, or can lead to unacceptable levels of mite damage.

It is apparent that the traditional philosophy of relying predominately on chemical mite control is inadequate. Alternative spider mite management practices which integrate a number of cultural, biological and chemical strategies are needed, and several alternatives have been studied. Pre-tassel applications of a selective miticide (propargite) are sometimes successful in reducing numbers of mites while having little negative impact on predators (Sloderbeck et al. 1985).

Natural suppression of spider mites has been considered as an alternative to traditional chemical control programs. Phytoseiid mites exotic to the western Great Plains were successfully (but not economically) employed by Pickett (1985) and Pickett and Gilstrap (1986) to suppress BGM populations. Several researchers have investigated the effects of a naturally occurring fungal pathogen, Neozygites sp., on populations of TSM in corn and peanuts in North Carolina (Brandenburg & Kennedy 1982a, 1982b, Boykin et al. 1984), and in cotton in Mississippi (Smith & Furr 1975). A naturally occurring fungal pathogen, Neozygites sp., of spider mites on corn has been observed for a number of years in the western Great Plains, but the pathogen/mite relationship has not been studied.

Purpose of this study. This study was part of a region-wide program investigating the biology, field ecology, and alternative management strategies of BGM and TSM in the western Great Plains. The pathogenic fungus, Neozygites sp., observed in the western Great Plains appears to play an important role in the life history of BGM and TSM, but very little is known about the relationship of the fungus to spider mite populations. The purpose of this study was to identify and describe the fungal pathogen infecting BGM and to study the field ecology of this fungus in the western Great Plains.

AREA DESCRIPTION

Western Great Plains. This study was conducted within a 128-km radius of Garden City. This area is representative of the western Great Plains, a geographic area that extends from the Texas panhandle to western Nebraska and from central Kansas and Nebraska west to the Front Range of the Rocky Mountains. The elevation of the area ranges from 610 to 1525 m.

Production of field corn in this area requires irrigation. The source of water is either pumping from the Ogallala Aquifer or irrigation canals supplied by the Arkansas River. Water is overhead- (center pivot), or surface- (gated pipe or siphon tube) applied.

The climate of the region is predominately middle latitude steppe. The eastern edge of the region merges with the humid continental zone, and is alternately affected by both climatic zones (Espenshade & Morrison, 1974). The growing season is typically hot and dry, with southerly winds during the day. Most precipitation occurs from March to October, often accompanying thunderstorms. Average annual rainfall for the region is 25 to 50 cm. The 30-year annual rainfall average (1951 to 1980) for the Garden City area was 45.5 cm, but average annual evaporation is 199 cm, leaving a considerable annual moisture deficit. The Garden City Experiment Station averages 66 days with measurable precipitation (Norwood 1983).

CHAPTER 2

IDENTIFICATION AND DESCRIPTION OF A PATHOGEN OF THE BANKS GRASS MITE AND THE TWOSPOTTED SPIDER MITE (ACARI: TETRANYCHIDAE): *NEOZYGITES* SP. (ENTOMOPHTHORALES: NEOZYGITACEAE)

SUMMARY

Major fungal epizootics periodically occur in populations of tetranychid mites in the western Great Plains of North America. Samples of the fungal pathogen were collected in Kansas and Texas. Short and long hyphal bodies, primary conidia, capillary conidiophores, capilliconidia, resting spores, and rhizoids typical of the genus Neozygites Witlaczil were described. Based on the surface texture of capilliconidia and resting spores, the shape of resting spores, and the occurrence of rhizoids, this fungus more closely resembles Neozygites adjarica than N. tetranychi or N. floridana. Because conidial dimensions overlap those fungi reported as N. floridana and N. tetranychi, and because the degree of morphological plasticity of members of this genus is not known, a more positive identification is not possible at this time.

INTRODUCTION

Major fungal epizootics in populations of the Banks grass mite (BGM), Oligonychus pratensis (Banks) and the twospotted spider mite (TSM), Tetranychus urticae Koch, have been observed periodically by field entomologists and agricultural consultants in the western Great Plains of North America. These epizootics are often associated with periods of cool, wet weather (Sloderbeck et al. 1985). In 1935, a fungal disease was reported to bring a serious infestation of TSM in elm trees under control in Kansas (Smith & Kelley 1936). A massive fungal epizootic was observed in corn spider mites in southwest Kansas in 1977, but no identification of the fungus was made (D. C. Cress and D. E. Mock, personal communication). A fungal pathogen from a sample of infected mites collected in southwest Kansas in 1982 was determined to resemble Neozygites adjarica (Tsintsadze & Vartapetov) (R. A. Humber, personal communication). A fungal pathogen from samples of infected BGM collected in the Texas Panhandle from 1981 to 1983 was identified as N. floridana (Pickett 1985, 1986a). In some cases the epizootics occurred in mite populations in research plots and the rapid decline in mite number interfered with interpretation of treatment effects (Buschman & Sloderbeck 1983a, 1983b).

Fungi in the genus Neozygites Witlaczil (1885) occur world-wide as pathogens of certain Homoptera (Witlaczil 1885, Le Ru 1986), Thysanoptera (Macleod et al. 1976, Keller & Wuest 1983), and Acari (Smitley 1985, Smitley et al. 1986a, 1986b). Three species infect tetranychid mites: Neozygites (=Entomophthora) floridana (Weiser &

Muma); Neozygites (=Triplosporium) tetranychi (Weiser); and Neozygites (=Entomophthora) adjarica (Tsintsadze & Vartapetov). Several authors have developed classification systems for the Entomophthorales and Entomophthoraceae (Batko 1964, MacLeod & Muller-Kogler 1973, Remaudiere & Keller 1980, Humber 1981, and Ben-Ze'ev & Kenneth 1982), but the diagnostic characters used at the specific level have failed to allow consistent separation of these three species (Humber et al. 1981).

Neozygites spp. have been identified and studied on tetranychid mites in other areas of North America (Smitley 1985, 1986a, 1986b), but to date there has been no attempt to study the pathogen of the BGM and TSM in the western Great Plains. Because these fungal epizootics play a major role in the population dynamics of spider mites (Smitley 1986 a, b), a better understanding of the relationship between the fungal pathogen and spider mites may lead to development of new mite management strategies. This study was undertaken to identify and describe the fungal pathogen infecting BGM in the western Great Plains.

MATERIALS AND METHODS

Fungus-infected BGM and TSM cadavers were collected on field corn (Kansas) and grain sorghum (Texas) leaves. The leaves were air dried and stored in paper bags inside 30-gallon plastic bags at 6°C. Calcium chloride was added to the plastic bags before sealing to reduce the humidity to about 70 percent.

The Kansas fungus was maintained in-vivo in BGM in the laboratory using a modification of Carner's (1976) and Smitley's (1985, 1986a) techniques. Cadavers that were not used immediately were stored at 5.5°C over calcium chloride in Parafilm® -sealed Petri dishes. The Texas fungus was induced to sporulate in order to view fresh primary conidia and capilliconidia, but no in-vivo culture was maintained.

Hyphal body morphology was determined in newly-infected BGM by sampling at intervals between infection and mummification. Fresh conidiophores, primary conidia, and capilliconidia were produced by placing mummified cadavers on cover slips in Petri dishes and incubating in a saturated atmosphere.

Measurements of fungal structures were made from semi-permanent lactophenol-mounted specimens. Care was taken to prevent distortion-producing pressure on the specimens, and structures that appeared distorted as a result of mounting procedure were not measured. Delicate structures such as young hyphal bodies were prepared for observation by gently dissecting cadavers in a drop of mounting media prior to adding the cover slip. Fungal nuclei were observed by preparing specimens in

2% aceto-orcein stain in place of semi-permanent media. Aceto-orcein mounts remained useable for several weeks.

Microscopic observations and measurements were made using an Olympus® BH/BHA compound light microscope with phase contrast attachment and an occular micrometer (100 to 400X). Photomicrographs were obtained using an Olympus®-equipped BH-2/BHS compound light microscope with BH-2-NIC differential interference contrast attachment (41 to 500X). Observation and transfer of live mites and mite-cadavers was facilitated by a Bausch & Lomb® dissecting microscope (10 to 70X).

Voucher specimens of Neozygites-infected BGM cadavers are preserved at the Herbarium ARSEF, USDA Insect Pathology Research Unit, Boyce Thompson Institute, Tower Rd., Ithaca, NY, (c/o R. A. Humber), and at the KSU Department of Entomology Scanning Electron Microscope Laboratory (c/o L. J. Krchma).

RESULTS AND DISCUSSION

General observations. The color of living infected mites ranged from near-normal to a faded-yellow. Some mites developed a yellowish, water-soaked appearance near the time of death. Following death, hyphal body-containing cadavers appeared light-beige to reddish-bronze but resting spore-containing cadavers appeared dark brown to black with a raspberry-like surface texture. Cadavers filled with resting spores were frequently firmly attached to the leaf surface by rhizoids.

Under dry conditions, hyphal body- or resting spore-filled cadavers appeared mummified and there was no further external change. Hyphal bodies in mummified cadavers remained viable for 10 months or more under cool, dry conditions in the laboratory. When subjected to a saturated atmosphere, hyphal body-filled cadavers attained a glass-beaded appearance in as few as two and one-half hours as emerging conidiophores produced primary conidia which in turn produced capillary conidiophores and capilliconidia.

Fungal infection occured when a mite made contact with a capilliconidium. The capilliconidium adhered to the mite integument by its terminal haptor, and a germ tube penetrated the integument at the point of attachment and the contents of the capilliconidium emptied into the mite body. Hyphal bodies first appeared within the mite body approximately 72 hours after initial contact with capilliconidia.

Hyphal bodies. Once infection occurred, hyphal body development in mites proceeded at ambient laboratory humidities. Short (Fig. 1hb) or long (Figs. 2hb, 3) hyphal bodies were observed, but not within the

same cadaver. Older short hyphal bodies and long hyphal bodies appeared light gray under transmitted tungsten or halogen light, but very young short hyphal bodies were nearly colorless and were better observed after staining or by using Nomarski interference contrast.

The morphology of short hyphal bodies was distinctly different from that of long hyphal bodies. Short hyphal bodies (Fig. 1hb) were quadrinucleate and tube-shaped with both ends rounded or with one end rounded and the other truncate, giving a distinct finger-shaped appearance. Short hyphal bodies measured 21.6 $\pm$ 1.2 µm by 6.7 $\pm$ 0.4 µm (Table 1). These measurements overlap similar measurements reported by other authors, except for Weiser (1968) who reports that hyphal bodies of N. tetranychi are longer and narrower than the Kansas/Texas fungus (Fig. 19a, 19b). Because some authors did not designate hyphal bodies short or long, it was necessary to do so before comparisons could be made. Where possible, the context of the discussion was used to assist in assigning hyphal body type.

Long hyphal bodies changed form as development progressed. Early stages were sinuous or elongate-digiform (Fig. 2). Later stages became clavate as a pyriform swelling developed at one end of each hyphal body (Fig. 3). Close packing of the swollen tips of long hyphal bodies against the inner surface of the mite integument often caused them to become angular (Fig. 3, top right). Long hyphal bodies were usually quadrinucleate. They were much longer and slightly narrower than short hyphal bodies, and often extended into mite legs. Nuclei sometimes appeared to migrate into the swollen ends of long hyphal bodies prior to conidiogenesis. Long hyphal bodies measured 52.8 $\pm$ 7.0 µm (both those

extending into mite legs and those restricted to the torso were measured) by 4.6 ± 0.4 µm (those clavate were measured at a point approximately equidistant from each end) (Table 1). These measurements appear to be significantly greater than the length of long hyphal bodies reported by other authors, except Carner (1976) (Fig. 20a). The width of Kansas laboratory-cultured long hyphal bodies are similar to those reported by Carner (1976), Humber et al. (1981), and Agudelo-Silva (1986), but are less than those reported for N. sp. nr. adjarica (Keller & Wuest 1983) or for N. floridana (Weiser & Muma 1966) (Fig. 20b).

Short hyphal bodies were unicellular but sometimes developed a distinct septum shortly before cell division (Fig. 1S). Long hyphal bodies were not observed to develop a septum (Figs. 2, 3). Short hyphal bodies appeared to be restricted to the mite torso, but long hyphal bodies were often observed extending into mite legs. No further external sign of fungal development occurred until the cadaver was subjected to a water-saturated atmosphere. When a hyphal body-filled cadaver was exposed to a saturated atmosphere, a single conidiophore developed from each long hyphal body, penetrated the mite integument, and gave rise to a single, unbranched, primary conidium (Figs. 4, 5). Cadavers which had sporulated previously in the field could be induced to sporulate again (although somewhat weakly) when subjected to a saturated atmosphere in the laboratory.

Primary conidia. Primary conidia were pyriform, usually quadrinucleate, smooth-walled, and appeared light gray under transmitted tungsten or halogen light (Fig. 6). The contents of primary conidia

often appeared granular-refringent. Most primary conidia were formed on the dorsal and lateral surfaces of cadavers. Primary conidia measured 15.2 ± 0.5 µm by 12.6 ± 0.4 µm (Table 1). As a group, the measurements of the Kansas/Texas primary conidia appear to be very similar to the measurements reported by others except for those reported for N. sp. nr. floridana Ramaseshiah (1971) (Figs. 21a, 21b). There appears to be a slight difference between the length and width of the field-collected and laboratory-cultured Kansas fungus (Figs. 21a, 21b). Near maturity, a septum formed between each conidiophore and primary conidium (Fig. 5S). When humidity remained high, primary conidia were forcibly ejected 1 to 3 mm away from the cadaver; a few were measured up to 9 mm away.

Primary conidia sometimes retained a remnant of the conidiophore or a droplet of cytoplasm and it appeared that this enabled the conidium to adhere to mite-webbing, glass, and plastic surfaces (Fig. 6R). Primary conidia were not observed to adhere to mites, though mites were subject to conidial showers and male mites were frequently observed attempting to copulate with sporulating female cadavers.

In a saturated atmosphere, each primary conidium (Figs. 5, 8PC) germinated to form one (usually) or more (occasionally) capillary conidiophores, each of which bore a single terminal capilliconidium (Fig. 8C). Occasionally, instead of producing a capillary conidiophore and capilliconidium, the primary conidium produced a broad germ tube which in turn produced a slightly smaller, nearly-identical, secondary conidium at the apex (Fig. 7). When capillary conidiophores were produced, the empty primary conidium remained attached as a hyaline

remnant (Figs. 8PC, 9C) and was observed in various degrees of inflation or deflation.

Capillary conidiophores and capilliconidia. Capillary conidiophores were long and very slender, always with an acute angle near the distal end (Fig. 8A, 9cap). The Kansas and Texas field-collected capillary conidiophores measured 44.9 ± 3.6 µm by 0.6 µm and 40.7 ± 3.4 µm by 0.6 µm, respectively (Table 1). These measurements were similar to the measurements reported by other authors, except for Weiser & Muma (1966) and Carner (1976) (Fig. 22). The development of capillary conidiophores was usually away from the surface on which conidia rested, suggesting that they function to raise the capilliconidia above the surface where they are more easily contacted by mites.

Capilliconidia (Fig. 10C) were almond-shaped, quadrinucleate, and always appeared smooth-walled. The color was light gray under transmitted tungsten or halogen light. The cell contents sometimes appeared granular-refringent. Kansas field-collected, Kansas laboratory-cultured, and Texas field-collected capilliconidia measured 18.9 ± 0.8 µm by 9.5 ± 0.2 µm, 18.2 ± 0.7 µm by 8.9 ± 0.4 µm, and 13.1 ± 1.6 µm by 8.6 ± 0.9 µm, respectively (Table 1). Capilliconidia that were detached from their capillary conidiophores were commonly observed to repeat production of nearly identical tertiary capillary conidiophores and capilliconidia (Fig. 9).

When mites were confined to a small leaf disk with sporulating cadavers they inevitably contacted suspended capilliconidia as they

moved about. On contact, capilliconidia became detached from from capillary conidiophores and adhered to the mite integument by a terminal haptor (Figs. 10H, 11). Most attached capilliconidia were observed on mite legs (Fig. 11L), but they were also found on other parts of the body. Germination and penetration of the host integument occurred at the point of attachment (Fig. 12). Capilliconidia were sometimes observed attached to setae on the legs and body of mites, but these apparently did not result in infection of the mite. In one case, a mite was observed to shed several capilliconidia by drawing its forelegs past its pepipalpi. This may be a means by which mites escape infection once contact has been made with capilliconidia.

Resting spores. Resting spores were double-walled and bi-nucleate (Fig. 13, 13N). They remained within the mite body and their presence was very conspicuous. Immature resting spores were spherical and hyaline to slightly yellow (Fig. 14IR). Immature resting spores were not measured. Mature resting spores were spherical to oval with a dark brown to black epispore (Fig. 15MR). Resting spore walls appeared to be smooth. The two nuclei sometimes appeared to be inclosed in a bladder-like structure. Dimensions of the Kansas field-collected, laboratory-cultured, and Texas field-collected resting spores were 19.7 $\pm$ 0.7 µm by 16.0 $\pm$ 0.6 µm, 19.9 $\pm$ 0.6 µm by 16.4 $\pm$ 0.8 µm, and 23.4 $\pm$ 0.7 µm by 17.1 $\pm$ 0.8 µm, respectively (Table 1). As a group, the lengths of the Kansas/Texas resting spores were similar to the lengths reported by other authors but when Kansas field-collected and laboratory-produced resting spores are compared to Texas field-collected and N. sp. nr.

adjarica (Keller & Wuest first accession 1983) resting spores, a length difference is apparent (Fig. 24a). As a group, the widths of the Kansas/Texas resting spores were similar to the widths reported by other authors, except for that of N. sp. nr. adjarica reported by Keller & Wuest (1983 first accession) (Fig. 24b).

Mature resting spores and short hyphal bodies were sometimes observed concurrently within the same mummified cadaver (Fig. 16HB, MR). Resting spores were very common in field-collected cadavers and often formed in laboratory-cultured cadavers. Resting spores are rare or absent in N. floridana and N. sp. nr. floridana (Weiser and Muma 1966, Carner & Canerday 1968, Ramaseshiah 1971, Kenneth et al. 1972, Humber et al. 1981, and Agudelo-Silva 1986). In Japan, no other fungal structures except resting spores occur in mites collected just before and during winter (Nemoto & Aoki 1975). Nemoto and Aoki (1975) hypothesize, based on their results and the conclusions of Kenneth et al. (1972), that resting spores have adaptive value as an overwintering mechanism, and that the minimum annual temperature at a given locality may be the determining factor in whether resting spores will form. In this study, resting spores were common at ambient field temperatures well before the colder temperatures of fall and winter occurred, and they were commonly formed in-vivo in the laboratory. In contrast to Nemoto & Aoki's (1975) observations, cadavers containing hyphal bodies were more numerous than cadavers containing resting spores two weeks before the first killing frost.

Rhizoids. Rhizoids emerged from the ventro-lateral surfaces of some cadavers that contained resting spores and firmly attached these cadavers to the leaf surface (Figs. 16,17). Rhizoids were observed only in cadavers that contained mature resting spores. It is possible that rhizoids developed from hyphal bodies occurring with the mature resting spores. Rhizoids were a light, smoky-gray color under tungsten or halogen light, and cell contents appeared refractile-granular. The apex of older rhizoids was slightly dilated. The leaf-rhizoid interface appeared smooth and shiny when a detached specimen was observed obliquely. Because rhizoids were extremely variable in length and it was difficult to determine their point of origin, they were not measured. In a laboratory study, structures resembling rhizoids developed within a few hours from resting spore-containing cadavers cultured in Grace's® Insect medium with or without 5% fetal bovine serum (technique: R. A. Humber, personal communication).

Rhizoids were common in both field-collected and laboratory-cultured cadavers in this study. Rhizoids are rare or absent (Weiser 1968, MacLeod & Muller-Kogler 1973, Waterhouse 1975, and Remaudiere & Keller 1980, and Humber 1981), except in those fungi identified as N. adjarica (Tsintsadze & Vartapetov 1976) and N. sp. nr. adjarica (Keller & Wuest 1983).

Taxonomic considerations. The Kansas/Texas fungus produced coenocytic (usually quadrinucleate) hyphal bodies and conidia and produced resting spores. These characters establish it as a member of the order Entomophthorales (Alexopoulos & Mims 1979). The Kansas/Texas

fungus had simple conidiophores and forcibly-discharged spherical to pyriform primary conidia. These characters establish it as a member of the family Entomophthoraceae (King & Humber 1981). The Kansas/Texas fungus may eventually prove to belong to the the new family, Neozygitaceae, recently proposed by Ben-Ze'ev (1986). The Kansas/Texas fungus had globose (pyriform), coenocytic (usually quadrinucleate) primary conidia and hyphal bodies, and produced two types of secondary conidia (one type a capilliconidium with a terminal haptor) and was collected in tetranychid mites. It had black or very dark brown, ovoid, and bi-nucleate resting spores. These characters establish it as a member of the genus Neozygites Witlaczil (Batko 1964, Remaudiere & Keller 1980, Humber 1981).

Identification to species is considerably more difficult. It is apparent that there are few differences between dimensions of the Kansas/Texas Neozygites spp. and the Neozygites spp. reported by other authors (Figs. 19 to 24). The most obvious size differences observed were that capilliconidia of the Kansas/Texas fungus were considerably shorter and narrower than the capilliconidia of N. tetranychi (Weiser 1968) and were considerably narrower than the capilliconidia of Neozygites sp. (Carner 1976) (Figs. 23a, 23b). Because there are so few differences in spore dimensions, we agree with Humber et al. (1981) that spore dimensions are not a good criteria for separation of N. floridana, N. tetranychi, and N. adjarica.

Some authors have used morphological features other than size as taxonomic criteria (MacLeod & Muller-Kogler 1973, Humber 1981, Ben-Ze'ev and Kenneth 1982), but these sometimes present other difficulties. One

such difficulty is illustrated by the inability to use the criterium of inflation/deflation of the empty primary conidial remnant (Fig. 8PC) (Humber et al. 1981) for the Kansas/Texas fungus, because all degrees of inflation/deflation were observed. Despite difficulties such as these, a few non-measurement morphological differences and similarities between the Kansas/Texas fungus and those observed by other authors are worthy of mention. The resting spores of N. tetranychi reported by Weiser (1968) have a ridged surface and are pyriform to almond-shaped, but the resting spores observed of the Kansas/Texas Neozygites sp. were always smooth and spherical to oval. Several authors have reported that the surface of capilliconidia of N. floridana and N. sp. near floridana is reticulated (MacLeod & Muller-Kogler 1973, Carner 1976) or striate (Weiser & Muma 1966), but the Kansas/Texas capilliconidia were always smooth whether or not attached to the capillary conidiophore (MacLeod & Muller-Kogler 1973).

Resting spores and rhizoids were commonly observed. This may or may not be taxonomically important. According to King and Humber (1981), presence of a particular spore type may be taxonomically significant, but absence of a particular spore may not be taxonomically significant. They also point out that environmental conditions in which the fungus develops and the fungal development stage at the time of observation (not fungal species as such), may determine structural morphology. If this is the case, the extensive overlap in structural dimensions (Figs. 19 to 24) may be explained in part by the varying environmental conditions encountered by the minimum of 10 mite species

hosted by a minimum of 15 plants in locations ranging from equatorial to 40 degrees north latitude (Appendix 1).

In comparing photographs of the Kansas/Texas Neozygites sp. (Figs. 1-18) with those of N. sp. nr. adjarica (Keller & Wuest 1983), it appears that the two fungi are very similar. The rhizoids presented by Keller & Wuest (1983, Fig. 24) are very similar to those presented in Fig. 18. The bi-nucleate resting spores presented by Keller & Wuest (1983, Fig. 25) are very similar to those presented in Fig. 13. The only apparent difference is that the resting spores of N. sp. nr. adjarica presented by Keller & Wuest (1983, Figs. 26 and 27) are slightly more pyriform than the Kansas/Texas fungus (Figs. 15, 16).

Because N. tetranychi has been observed only once (Weiser 1968), and N. adjarica and N. sp. nr. adjarica (Tsintsadze & Vartapetov 1976 and Keller & Wuest 1983, respectively) have been observed only twice, the descriptions and material available for comparison are limited. As the three species become better-known, there is a possibility that two of the three species may be relegated to synonomy, leaving only a single morphologically plastic species. (R. A. Humber, personal communication).

It appears that a positive specific identification of the Kansas/Texas fungus may be difficult or impossible at this time. However, based on the surface texture of capilliconidia and resting spores, the common presence of rhizoids and resting spores, prior collection of fungal material tentatively identified as N. sp. near adjarica (R. A. Humber personal communication) in southwest Kansas, it appears that the Kansas/Texas fungus more closely resembles the N. adjarica of Tsintsadze & Vartapetov (1976) or the N. sp. nr adjarica

of Keller & Wuest (1983) than either of the other two Neozygites sp. pathogenic in populations of tetranychid mites. A more definite conclusion awaits further knowledge about the three Neozygites spp. infecting spider mites.

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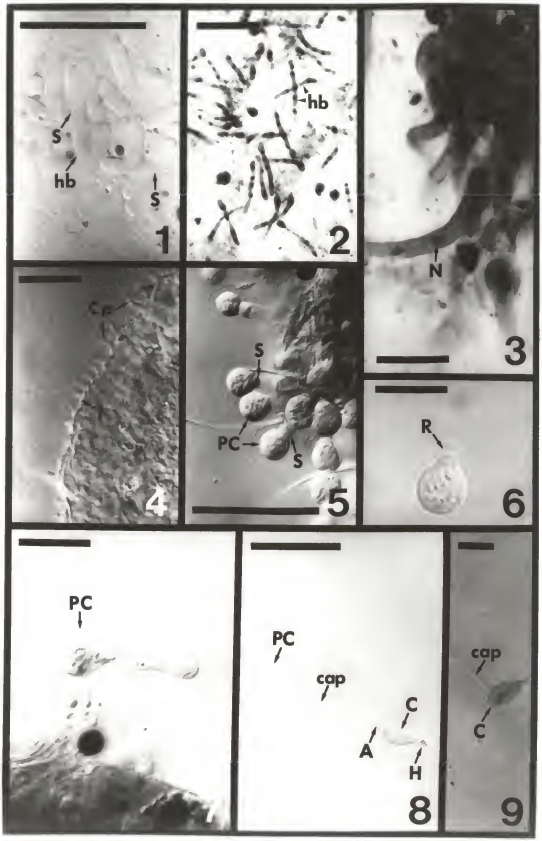
Table 1. Dimensions (μm) of key fungal structures.

Fungal Structure	Length $\pm$ $s_x(n)$	Width $\pm$ $s_x(n)$
Primary Conidia		
Kansas (field)	15.2 $\pm$ 0.5(23)	12.6 $\pm$ 0.4(23)
Kansas (lab)	13.7 $\pm$ 0.6(21)	11.2 $\pm$ 0.5(21)
Texas (field)	14.2 $\pm$ 0.5(23)	11.7 $\pm$ 0.5(23)
Capilliconidia		
Kansas (field)	18.9 $\pm$ 0.8(17)	9.5 $\pm$ 0.2(17)
Kansas (lab)	18.2 $\pm$ 0.7(21)	8.9 $\pm$ 0.4(21)
Texas (field)	13.1 $\pm$ 1.6(23)	8.5 $\pm$ 0.9(23)
Resting Spores		
Kansas (field)	19.7 $\pm$ 0.7(23)	15.6 $\pm$ 0.6(23)
Kansas (lab)	19.9 $\pm$ 0.6(21)	16.4 $\pm$ 0.8(21)
Texas (field)	23.4 $\pm$ 0.7(23)	17.2 $\pm$ 0.8(23)
Capillary Conidiophores		
Kansas (field)	44.9 $\pm$ 3.7(19)	0.6[1]
Texas (field)	40.7 $\pm$ 1.6(23)	0.6[1]
Short Hyphal Bodies		
Kansas (lab)	21.6 $\pm$ 1.2(21)	6.7 $\pm$ 0.4(21)
Long Hyphal Bodies		
Kansas (lab)	52.8 $\pm$ 7.0(21)	4.6 $\pm$ 0.4(7)

[1]Width was below resolution of the optic micrometer.

Figures 1 to 9: Stages of Development of N. sp. near adjarica in BGM. Fig 1. Short hyphal bodies showing septa (S) prior to cell division. Fig. 2. Early appearance of long hyphal bodies (hb). Fig. 3. Late appearance of long hyphal bodies (hb) showing nuclei (N). Figs. 4. Penetration of integument (I) by conidiophores (Cp). Fig. 5. Formation of primary conidia (PC) showing septa (S) between conidia and conidiophores. Fig. 6. Primary conidium showing conidiophore remnant or cytoplasm droplet (R). Fig. 7. Primary conidium (PC) in the process of forming a secondary conidium (rare). Fig. 8. Primary conidium (PC) forming a single capillary conidiophore (cap) and capilliconidium (C). Note angle (A) of capillary conidiophore and haptor (H) at apex of capilliconidium. Fig. 9. Tertiary capillary conidiophore (cap) arising from capilliconidium (C). (Bars on Figs. 1, 2, 4, 5, and 8 correspond to 50 µm. Bars on Figs. 3, 6, 7, and 9 correspond to 20 µm.)


S
hb
S
1
hb
2
N
3
Cp
4
S
PC
S
5
R
6
PC
PC
cap
C
A
H
8
cap
C
9

Figures 10 to 18: Stages of development of N. sp. near adjarica in BGM, continued. Fig. 10. Capilliconidium (C) with terminal haptor (H). Fig. 11. Capilliconidia (C) adhering to integument of a mite leg (L). Fig. 12. Early penetration (white arrow) of mite integument by germ tube from a capilliconidium. Fig. 13. Young resting spores showing double wall and two nuclei (N). Fig. 14. Immature resting spores (IR) within male BGM. Fig. 15. Mature resting spores (MR) within a BGM nymph. Fig. 16. Simultaneous occurrence of short hyphal bodies (hb) and mature resting spores (MR). Fig. 17. Early rhizoid (rhi) development. 18. Rhizoid (rhi) emergence through mite integument (I). (Bars on Figs. 10, 11, 14, 15, 16, and 17 correspond to 50 μm. Bars of Figs, 12, 13, and 18 correspond to 20 μm.)

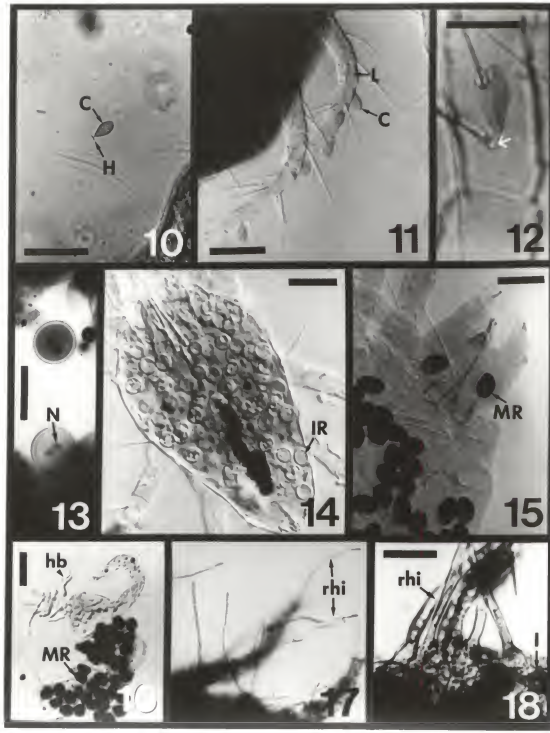

C
H
10
L
C
11
12
N
13
IR
14
MR
15
hb
MR
16
rhi
17
rhi
18

Figure 19. Dimensions (um) of various Neozygites spp. short hyphal bodies. a. Length. b. Width

Legend:

Narrow vertical bar = range, where reported.

Wide vertical bar = 95% C.I., where reported.

Horizontal bar = mean, where reported.

KSF = Kansas field-collected.

KSL = Kansas laboratory-cultured.

TX = Texas field-collected.

A = adjarica (Tsintsadze & Vartapetov 1976).

NrA1 = sp. near adjarica (Keller & Wuest 1983).

NrA2 = sp. near adjarica (Keller & Wuest 1983).

T = tetranychi (Weiser 1968).

F1 = floridana (Weiser & Muma 1966).

F2 = floridana (Nemoto & Aoki 1975).

F3 = floridana (Kenneth et al. 1972).

NrF = sp. near floridana (Rameseshiah 1971).

Sp1 = species not designated (Carner 1976).

Sp2 = species not designated (Humber et al. 1981).

Sp3 = species not designated (Agudelo-Silva 1986).

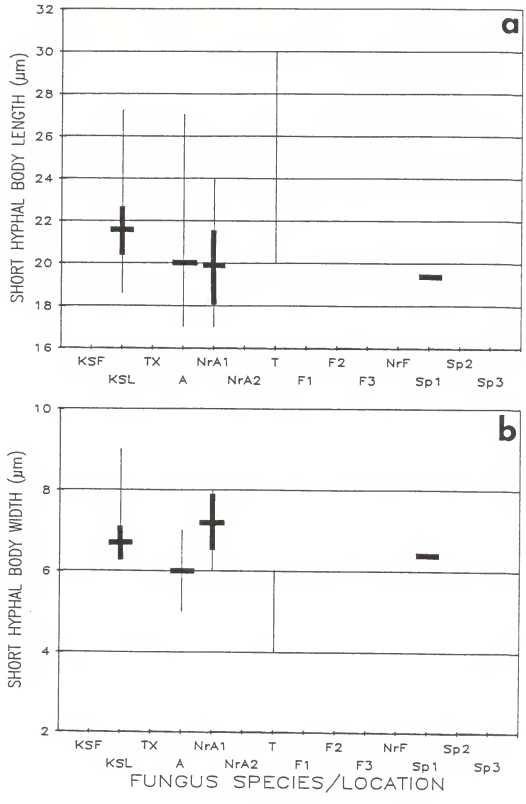

a
SHORT HYPHAL BODY LENGTH (µm)
32
30
28
26
24
22
20
18
16
KSF
KSL
TX
A
NrA1
NrA2
T
F1
F2
F3
NrF
Sp1
Sp2
Sp3
b
SHORT HYPHAL BODY WIDTH (µm)
10
8
6
4
2
FUNGUS SPECIES/LOCATION

Figure 20. Dimensions (um) of various Neozygites spp. long hyphal bodies. a. Length. b. Width.

Legend:

Narrow vertical bar = range, where reported.

Wide vertical bar = 95% C.I., where reported.

Horizontal bar = mean, where reported.

KSF = Kansas field-collected.

KSL = Kansas laboratory-cultured.

TX = Texas field-collected.

A = adjarica (Tsintsadze & Vartapetov 1976).

NrA1 = sp. near adjarica (Keller & Wuest 1983).

NrA2 = sp. near adjarica (Keller & Wuest 1983).

T = tetranychi (Weiser 1968).

F1 = floridana (Weiser & Muma 1966).

F2 = floridana (Nemoto & Aoki 1975).

F3 = floridana (Kenneth et al. 1972).

NrF = sp. near floridana (Rameseshiah 1971).

Sp1 = species not designated (Carner 1976).

Sp2 = species not designated (Humber et al. 1981).

Sp3 = species not designated (Agudelo-Silva 1986).

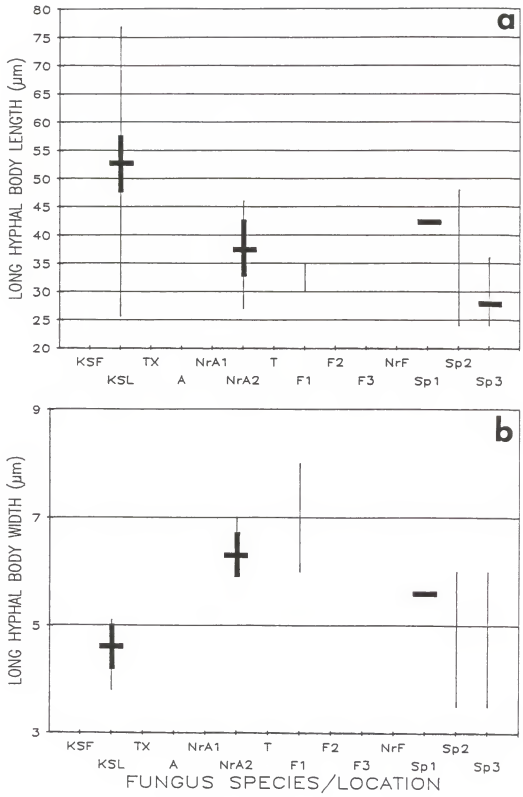

a
LONG HYPHAL BODY LENGTH (µm)
80
75
70
65
60
55
50
45
40
35
30
25
20
KSF
KSL
TX
A
NrA1
NrA2
T
F1
F2
F3
NrF
Sp1
Sp2
Sp3
b
LONG HYPHAL BODY WIDTH (µm)
9
7
5
3
FUNGUS SPECIES/LOCATION

Figure 21. Dimensions (um) of various Neozygites spp. primary conidia. a. Length. b. Width.

Legend:

Narrow vertical bar = range, where reported.

Wide vertical bar = 95% C.I., where reported.

Horizontal bar = mean, where reported.

KSF = Kansas field-collected.

KSL = Kansas laboratory-cultured.

TX = Texas field-collected.

A = adjarica (Tsintsadze & Vartapetov 1976).

NrA1 = sp. near adjarica (Keller & Wuest 1983).

NrA2 = sp. near adjarica (Keller & Wuest 1983).

T = tetranychi (Weiser 1968).

F1 = floridana (Weiser & Muma 1966).

F2 = floridana (Nemoto & Aoki 1975).

F3 = floridana (Kenneth et al. 1972).

NrF = sp. near floridana (Rameseshiah 1971).

Sp1 = species not designated (Carner 1976).

Sp2 = species not designated (Humber et al. 1981).

Sp3 = species not designated (Agudelo-Silva 1986).

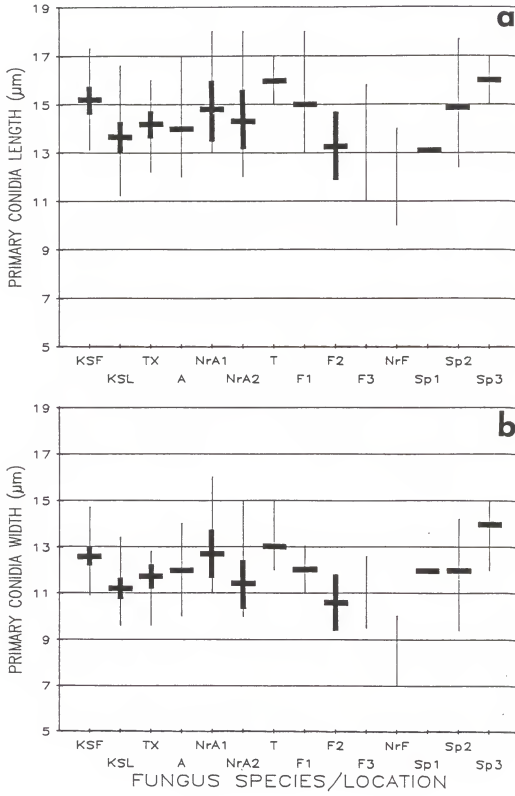

a
PRIMARY CONIDIA LENGTH (µm)
19
17
15
13
11
9
7
5
KSF
KSL
TX
A
NrA1
NrA2
T
F1
F2
F3
NrF
Sp1
Sp2
Sp3
b
PRIMARY CONIDIA WIDTH (µm)
19
17
15
13
11
9
7
5
KSF
KSL
TX
A
NrA1
NrA2
T
F1
F2
F3
NrF
Sp1
Sp2
Sp3
FUNGUS SPECIES/LOCATION

Figure 22. Length (um) of various Neozygites spp. capillary conidiophores.

Legend:

Narrow vertical bar = range, where reported.

Wide vertical bar = 95% C.I., where reported.

Horizontal bar = mean, where reported.

KSF = Kansas field-collected.

KSL = Kansas laboratory-cultured.

TX = Texas field-collected.

A = adjarica (Tsintsadze & Vartapetov 1976).

NrA1 = sp. near adjarica (Keller & Wuest 1983).

NrA2 = sp. near adjarica (Keller & Wuest 1983).

T = tetranychi (Weiser 1968).

F1 = floridana (Weiser & Muma 1966).

F2 = floridana (Nemoto & Aoki 1975).

F3 = floridana (Kenneth et al. 1972).

NrF = sp. near floridana (Rameseshiah 1971).

Sp1 = species not designated (Carner 1976).

Sp2 = species not designated (Humber et al. 1981).

Sp3 = species not designated (Agudelo-Silva 1986).

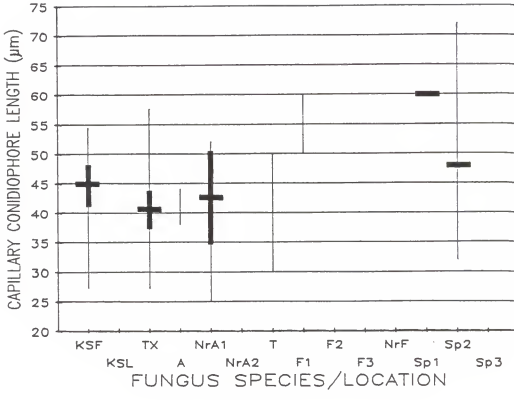

CAPILLARY CONIDIOPHORE LENGTH (µm)
75
70
65
60
55
50
45
40
35
30
25
20
KSF
KSL
TX
A
NrA1
NrA2
T
F1
F2
F3
NrF
Sp1
Sp2
Sp3
FUNGUS SPECIES/LOCATION

Figure 23. Dimensions (um) of various Neozygites spp. capilliconidia.

a. Length. b. Width.

Legend:

Narrow vertical bar = range, where reported.

Wide vertical bar = 95% C.I., where reported.

Horizontal bar = mean, where reported.

KSF = Kansas field-collected.

KSL = Kansas laboratory-cultured.

TX = Texas field-collected.

A = adjarica (Tsintsadze & Vartapetov 1976).

NrA1 = sp. near adjarica (Keller & Wuest 1983).

NrA2 = sp. near adjarica (Keller & Wuest 1983).

T = tetranychi (Weiser 1968).

F1 = floridana (Weiser & Muma 1966).

F2 = floridana (Nemoto & Aoki 1975).

F3 = floridana (Kenneth et al. 1972).

NrF = sp. near floridana (Rameseshiah 1971).

Sp1 = species not designated (Carner 1976).

Sp2 = species not designated (Humber et al. 1981).

Sp3 = species not designated (Agudelo-Silva 1986).

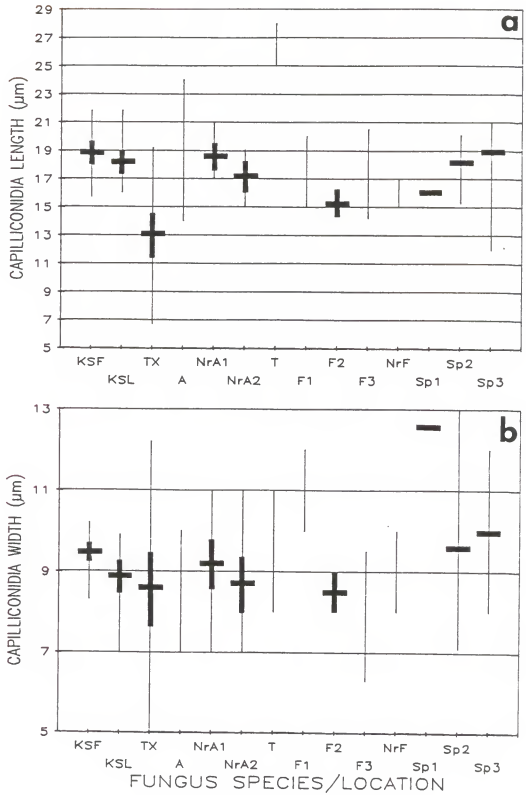

a
CAPILLICONIDIA LENGTH (µm)
29
27
25
23
21
19
17
15
13
11
9
7
5
KSF
KSL
TX
A
NrA1
NrA2
T
F1
F2
F3
NrF
Sp1
Sp2
Sp3
b
CAPILLICONIDIA WIDTH (µm)
13
11
9
7
5
KSF
KSL
TX
A
NrA1
NrA2
T
F1
F2
F3
NrF
Sp1
Sp2
Sp3
FUNGUS SPECIES/LOCATION

Figure 24. Dimensions of various Neozygites spp. resting spores.

a. Length. b. Width.

Legend:

Narrow vertical bar = range, where reported.

Wide vertical bar = 95% C.I., where reported.

Horizontal bar = mean, where reported.

KSF = Kansas field-collected.

KSL = Kansas laboratory-cultured.

TX = Texas field-collected.

A = adjarica (Tsintsadze & Vartapetov 1976).

NrA1 = sp. near adjarica (Keller & Wuest 1983).

NrA2 = sp. near adjarica (Keller & Wuest 1983).

T = tetranychi (Weiser 1968).

F1 = floridana (Weiser & Muma 1966).

F2 = floridana (Nemoto & Aoki 1975).

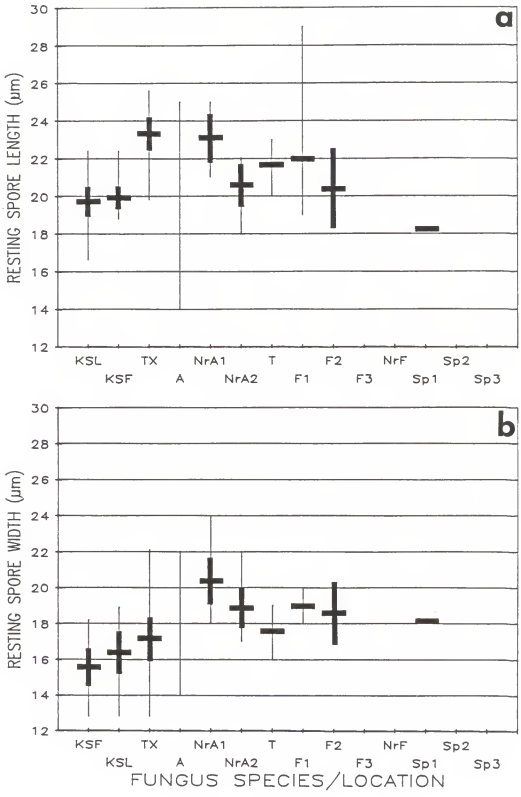
F3 = floridana (Kenneth et al. 1972).

NrF = sp. near floridana (Rameseshiah 1971).

Sp1 = species not designated (Carner 1976).

Sp2 = species not designated (Humber et al. 1981).

Sp3 = species not designated (Agudelo-Silva 1986).


a
RESTING SPORE LENGTH (µm)
30
28
26
24
22
20
18
16
14
12
KSL
KSF
TX
A
NrA1
NrA2
T
F1
F2
F3
NrF
Sp1
Sp2
Sp3
b
RESTING SPORE WIDTH (µm)
30
28
26
24
22
20
18
16
14
12
KSF
KSL
TX
A
NrA1
NrA2
T
F1
F2
F3
NrF
Sp1
Sp2
Sp3
FUNGUS SPECIES/LOCATION

APPENDIX 1

The following tables present a comprehensive summary of the current study and literature reports of those Neozygites sp. pathogenic on plant-feeding spider mites: Neozygites species, reporting author, collection location, host mite, host plant, measurement reporting format, and structural dimensions of various fungal life stages are reported.

		CURRENT STUDY			LITERATURE REPORTS			
NEOZYGITES SPECIES		nr. adjarica	nr. adjarica	nr. adjarica	adjarica	nr. adjarica	nr. adjarica	tetranychi
AUTHOR		CURRENT STUDY	CURRENT STUDY	CURRENT STUDY	Tsint. & Vart. (1976)	Keller & Wuest I (1983)	Keller & Wuest II (1983)	Welser (1968)
LOCATION		KS, USA (Field)	KS, USA (Lab)	TX, USA (Field)	USSR	Switzerland	Switzerland	Czechoslovakia
HOST MITE		O. pratensis	O. pratensis	O. pratensis	T. urticae	T. urticae	T. urticae	T. althease
HOST PLANT		Field Corn	Field Corn	Grain Sorghum	Not Reported	Green Beans	Green Beans	Pome Fruits
REPORTING FORMAT*		(b)	(b)	(b)	(c,d)	(a)	(a)	(c,e)
STRUCTURAL DIMENSIONS	SHORT HYPHAL BODIES (µm) L	- - -	21.6 + 1.2(21)	- - - -	17.0-27.0,20.0	19.9 + 1.9(25)	- - -	20.0-30.0
	W	- - -	6.7 + 0.4(21)	- - -	5.0- 7.0, 6.0	7.2 + 0.7(25)	- - -	4.0- 6.0
	LONG HYPHAL BODIES (µm) L	- - -	52.8 + 7.0(21)	- - -	- - -	- - -	37.3 + 5.3(25)	- - -
	W	- - -	4.6 + 0.4 (7)	- - -	- - -	- - -	6.3 + 0.5(25)	- - -
	PRIMARY CONIDIA (µm) L	15.2 + 0.5(23)	13.7 + 0.6(21)	14.2 + 0.5(23)	12.0-17.0,14.0	14.8 + 1.2(50)	14.3 + 1.1(50)	15.0-17.0,16.0
	W	12.6 + 0.4(23)	11.2 + 0.5(21)	11.7 + 0.5(23)	10.0-14.0,12.0	12.7 + 1.0(50)	11.4 + 1.0(50)	12.0-15.0,13.0
	CAPILLARY CONIDIOPHORES (µm) L	45.0 + 3.7(19)	- - -	40.7 + 3.4(23)	- - -	42.7 + 6.1(25)	- - -	30.0-50.0
	W	0.6 + 0.0(19)	- - -	0.6 + 0.0(23)	- - -	- - -	- - -	1.0
	CAPILLICONIDIA (µm) L	19.0 + 0.8(17)	18.2 + 0.7(21)	13.0 + 1.6(23)	14.0-24.0	18.7 + 0.9(50)	17.2 + 1.2(50)	25.0-28.0
	W	9.5 + 0.2(17)	8.9 + 0.4(21)	8.6 + 0.9(23)	7.0-10.0	9.2 + 0.8(50)	8.7 + 0.8(50)	8.0-11.0
	RESTING SPORES (µm) L	19.7 + 0.7(23)	19.9 + 0.6(21)	23.4 + 0.7(23)	14.0-25.0	23.1 + 1.3(50)	20.6 + 0.8(50)	20.0-23.0
	W	16.0 + 0.6(23)	16.4 + 0.8(21)	17.1 + 0.8(23)	14.0-24.0	20.4 + 1.4(50)	18.9 + 1.1(50)	16.0-19.0

*Measurement reporting format is: (a). $\bar{X} + s_{\bar{X}}(n)$ (standard deviation)
(b). $\bar{X} + (t_{.05} \times s_{\bar{X}})(n)$ (95% CL: Snedecor & Cochran 1967, Little & Hills 1972)
(c). Range
(d). Range, $\bar{X}(n)$
(e). $\bar{X}$

		LITERATURE REPORTS, CONTINUED						
NEOZYGITES SPECIES		floridana	floridana	floridana	nr. floridana	(species)	(species)	(species)
AUTHOR		Weiser & Muma (1966)	Nemoto & Aoki (1975)	Kenneth et al. (1972)	Ramaseshiah (1971)	Carner (1976)	Humber et al. (1981)	Agudelo-Silva (1986)
LOCATION		FL, USA	Japan	Israel	India	AB & SC, USA	Brazil	Venezuela
HOST MITE		Eut. banksii	O. hondoensis	T. urticae	Tetranychidae	T. urticae	T. evansi	M. progressivus
HOST PLANT		Citrus	Japanese Cedar	Castor bean	6 dif. host	Cotton	Tomato	Cassava
REPORTING FORMAT*		(c, d, e)	(a)	(c)	(c)	(c, e)	(d)	(a)
STRUCTURAL OMENS	SHORT HYPHAL BODIES (µm) L	- - -	- - -	- - -	- - -	19.4-31.9	24.0-48.0	28.0 + 4.3(30)
	W	- - -	- - -	- - -	- - -	5.6- 6.4	3.5- 6.0	8.0 + 0.8(30)
	LONG HYPHAL BODIES (µm) L	30.0-35.0	- - -	- - -	- - -	42.3	- - -	- - -
	W	6.0- 8.0	- - -	- - -	- - -	5.6	- - -	- - -
	PRIMARY CONIDIA (µm) L	13.0-18.0,15.0	13.3 + 1.4(NA)	11.0-15.8	10.0-14.0	13.1	12.4-17.7,14.9(75)	16.4 + 1.5(7)
	W	11.0-13.0,12.0	10.6 + 1.2(NA)	9.5-12.6	7.0-10.0	12.0	9.4-14.2,12.0(75)	14.0 + 1.1(7)
	CAPILLARY CONIDIOPHORES (µm) L	50.0-80.0,	- - -	- - -	- - -	60.8	32.0-72.0,48.0(50)	- - -
	W	1.5	- - -	- - -	- - -	- - -	1.0- 2.0	- - -
	CAPILLICONIDIA (µm) L	15.0-20.0	15.3 + 0.9(NA)	14.2-20.5	15.0-17.0	16.1	15.3-20.1,18.2(50)	19.0 + 1.8(14)
	W	10.0-12.0	8.5 + 0.5(NA)	6.3- 9.5	8.0-10.0	12.6	7.1-13.0, 9.6(50)	10.0 + 1.3(14)
	RESTING SPORES (µm) L	19.0-29.0,22.0	20.4 + 2.1(NA)	- - -	- - -	- - -	- - -	- - -
	W	18.0-20.0,19.0	18.6 + 1.7(NA)	- - -	- - -	18.2	- - -	- - -

*Measurement reporting format is: (a). $\bar{X} + s_{\bar{X}}(n)$ (standard Deviation)
(b). $\bar{X} + (t_{.05} \times s_{\bar{X}})(n)$ (95% CL: Snedecor & Cochran 1967, Little & Hills 1972)
(c). Range
(d). Range, $\bar{X}(n)$
(e). $\bar{X}$

**Capilliconidia length measured to proximal end of isthmus bearing haptor.

CHAPTER 3

FIELD ECOLOGY OF A FUNGAL PATHOGEN OF *OLIGONYCHUS PRATENSIS* AND *TETRANYCHUS URTICAE* (ACARI: TETRANYCHIDAE) IN FIELD CORN: *NEOZYGITES* SP. (ENTOMOPHTHORALES: NEOZYGITACEAE)

ABSTRACT

Corn fields were monitored for the effects of a fungal spider mite pathogen, Neozygites sp., on populations of Oligonychus pratensis and Tetranychus urticae. The related factors of relative humidity and precipitation, predatory and incidental arthropods, and the effects of insecticides and miticides were also monitored with respect to their overall effect on the mite-pathogen relationship. There is a complex combination of interactions among these factors. Several fungal epizootics were observed, apparently coinciding with mid-August or later periods of 8 to 10 hours per day of $\geq$ 80% ambient relative humidity. No fungal epizootics were observed earlier than mid-August even when these periods of relative humidity occurred. At only one study site did it appear that the fungus was the major mortality agent in 1984 and 1985. This is in contrast to prior field observations which indicated that Neozygites sp. was capable of decimating spider mite populations. In this study, combinations of factors other than Neozygites sp., including predatory arthropods, weather, and possibly host-plant senescence, appeared to be the most important agents of mortality. Insecticide and miticide applications appeared to have a much greater impact on predatory and incidental arthropods than on spider mites. Though Neozygites sp. appeared to have no regularly occurring negative impact on numbers of spider mites, it appeared to be one of a combination of factors that, as a whole, are capable of suppressing spider mite populations. Those who monitor spider mites or conduct spider mite

research should be aware of the potential impact of Neozygites sp. on spider mite populations.

INTRODUCTION

Dramatic, naturally-occurring, fungal epizootics of the Banks grass mite (BGM), Oligonychus pratensis (Banks) and the twospotted spider mite (TSM), Tetranychus urticae Koch have been reported periodically by entomologists and agricultural consultants in the western Great Plains of North America. One such epizootic was observed in southwest Kansas in 1977 but the pathogen was not identified (D. E. Mock and D. C. Cress personal communication). A fungal pathogen collected from infected mites in southwest Kansas field corn, Zea mays L., in 1982 closely resembled Neozygites adjarica (Tsintsadze & Vartapetov), but because of taxonomic difficulties, a more positive identification was not possible (R. A. Humber personal communication). In 1982, Neozygites sp. caused high spider mite mortality in experimental plots, which interfered with interpretation of treatment effects (Buschman & Sloderbeck 1983a, b). Pickett (1985) lists N. floridana (Weiser & Muma) among natural enemies of BGM collected in the Texas panhandle from 1981 to 1983.

The genus Neozygites Witlaczil (1885) includes species pathogenic in populations of certain Homoptera (Witlaczil 1885, Le Ru 1986), Thysanoptera (MacLeod et al. 1976, Keller & Wuest 1983), and Acari (Smitley 1985, Smitley et al. 1986a, 1986b). A closely related group of three species, N. floridana (Weiser & Muma 1966), N. tetranychi (Wieser 1968), and N. adjarica (Tsintsadze & Vartapetov 1976) have been reported to infect at least nine tetranychid mite species throughout the world (Remaudiere & Keller 1980, Smitley et al. 1986a). It is very

difficult to separate these three species with the taxonomic tools available and there is no consensus on their systematic status (Humber 1981, Humber et al. 1981). They may be members of a single morphologically plastic species (R. A. Humber, personal communication).

Reports of Neozygites spp. epizootic in populations of tetranychid mites are summarized by Smitley (1985), and Smitley et al. (1986a, 1986b). In the United States, most studies of Neozygites sp. epizootics have been conducted in the southeastern states and in California. Several studies have been conducted on the effects of Neozygites spp. on populations of TSM in corn and peanuts in North Carolina (Smitley 1986a, b), and in cotton in Mississippi (Smith and Furr 1975). Most researchers feel that Neozygites spp. play a major role in natural suppression of tetranychid mite populations (Smitley 1986b).

To date, there have been no studies of the field ecology of Neozygites sp. infecting BGM and TSM in the western Great Plains, and there have been no studies of the relationship of Neozygites spp. to populations of BGM. The purpose of this study was to determine the importance of Neozygites sp. in the population dynamics of corn spider mites in the western Great Plains.

MATERIALS AND METHODS

1984 and 1985 General.

Field description. Six commercial corn fields (2- to 72-ha) within a 128-km radius of Garden City, Kansas were selected for sampling in 1984 and 1985. Ten of these 12 fields were privately-owned and two were on the Garden City Experiment Station (GCX). Fields were selected either on the basis of a history of spider mite infestation or the presence of an early-season infestation.

Finney County A (FIA) was located in the Arkansas river flood plain 2 km south and 3.5 km east of Deerfield, KS, was furrow-irrigated, and was studied in 1984 and 1985. Finney County B (FIB) was located 8 km east and 2.4 km north of Garden City, KS, was furrow-irrigated, and was studied only in 1985. Finney County C (FIC) was located 10 km north of Garden City, was furrow irrigated, and was studied for part of 1985. Sampling was discontinued in early August. Finney County D (FID) was located 5 km east of Garden City, was center pivot-irrigated, and was studied only in 1984. Finney County E (FIE) was located 5 km north and 2 km west of Holcomb, KS, was furrow irrigated, and was studied only in 1984. Stanton County A (STA) and B (STB) were located 19 km north-northeast of Johnson City, KS. STB was center pivot-irrigated and was studied only in 1984. STA was furrow-irrigated and was studied in 1984 and 1985. Ford County A (FOA) was located in the Arkansas river flood plain 13 km east-southeast of Dodge City, KS , was furrow-irrigated, and was studied in 1984 and part of 1985. Sampling was discontinued in early August. Haskell County A (HAA) was located 26 km south of

Pierceville, KS, was furrow-irrigated, and was studied only in 1985. Study sites at FIA, STB, and FOA were in corn adjacent to documented mite-overwintering habitat (Buschman and Dick in press) and they had a history of moderate to severe mite infestations. Study sites at STA, HAA, FIB, and FIC were in corn adjacent to suspected mite-overwintering habitat, and had a history of moderate to severe mite infestations. Haskell County A had a seven year history of very severe mite infestations. Study sites at FID and FIE were in corn isolated from known overwintering habitat (Buschman and Dick, in press) and occasionally developed mite infestations. In 1984 all fields contained four study sites, except for FIC which contained two study sites. In 1985 all fields contained two study sites, except for FIB which contained one study site.

Because all fields were commercial fields, they were subject to pesticide applications. No record of pesticide treatments was kept in 1984 though most fields were treated for corn borers and all fields were treated for spider mites. In 1985 only FIA and STA were treated for corn borers, but all six fields were treated for spider mites (Table 2).

Weather data collection. Crop canopy temperature and humidity data were collected at FIA and STA in both 1984 and 1985 using Weathertronics® or Cole Parmer® hygrothermographs placed 1 m above the soil surface in standard weather shelters. The weather data for both years was incomplete, often at critical periods, so data from the automated GCX and manually-read National Oceanic and Atmospheric Administration (NOAA) recording stations at four widely-spaced southwest Kansas sites were used. When canopy relative humidity ((hours per day $\geq$

90% at FIA (SE)) was plotted against ambient relative humidity (hours per day $\geq$ 80% at GCX) (34 km apart), a reasonable correlation ($r = 0.83$, $P < 0.001$) was obtained. This allowed substitution of ambient hours per day $\geq$ 80% for canopy hours per day $\geq$ 90% for purposes of this study. The canopy relative humidity (hours per day $\geq$ 90%) has been used by other authors to associate relative humidity with the development of Neozygites epizootics in populations of spider mites and aphids (Missioner 1970, Brandenburg & Kennedy 1982b, Smitley 1986a).

1984 Procedure.

Field samples. Sample sites were located either 50 m X 20 rows in from field corners used as reference points, or were 20 m (or 20 rows) in from marked plants on the edge of circular fields. Weekly samples consisting of 10 to 15 spider mite-infested leaves were systematically pulled or cut from plants at each study site. Leaves selected ranged from the lowest live leaf to the ear-leaf. The leaves were folded into 4-1 plastic bags and packed in an ice chest for transport to the laboratory. Samples were refrigerated at 4 to 6°C for two weeks to allow fungal development or to induce resting spore formation in infected mites (Weiser 1968, Nemoto & Aoki, 1975) and were then frozen for future evaluation. Adult female mites were visually counted on two to six half-plants, depending on mite numbers and distribution pattern. Mite samples for species identification were collected during the visual counts using a hand-held aspirator and 2-dram vials containing 70% methanol. Mite predators and other arthropods associated with mite colonies were not monitored in 1984.

Vial evaluation. Vials were evaluated by systematically subsampling and mounting mites in Hoyer's medium. Mounts were microscopically examined (Olympus ® BH/BHA equipped with phase-contrast) and the proportion of each mite species was determined based on the presence (BGM) or absence (TSM) of the empodial claw (Krantz 1978). The numbers of each mite species per plant were calculated from visual counts adjusted by the ratio of BGM to TSM.

Leaf sample evaluation. The frozen leaves were thawed, cut into 20- to 23-cm sections, and air-dried until no free moisture remained. Leaf sections were numbered and a random subsample was selected. Random one-field-of-vision (25X, Bausch & Lomb ® dissecting microscope) sites along the midrib of the base- and mid-section on the underside of each leaf were examined for the presence of spider mites. Spider mites were counted, sub-samples were mounted in Hoyer's medium, and mounts were microscopically (Olympus ® BH/BHA equipped with phase-contrast) examined for fungal infection. A mite was considered infected if it contained hyphal bodies or resting spores, or had capilliconidia attached to its integument.

1985 Procedure.

Use of the leaf brushing machine. To improve labor efficiency, reduce the time between field-sampling and laboratory evaluation, and to eliminate problems with saprophytic fungi that occurred in 1984, a method of estimating spider mite populations using a modified leaf-brushing machine was developed (Henderson & McBurnie 1943, Morgan et al. 1955). The brushing machine was modified by removing the driven brush

in order to reduce leaf-shredding, crushing of mites, and clumping of mites and mite eggs in mite webbing.

Field samples. Study sites were sampled weekly. Study sites were 80 m by 10 rows in from field corners used as reference points. Weekly samples consisted of two sets of two plants systematically selected from each study site as follows; the first plant was selected with eyes closed, and then three more plants were selected by collecting every ninth plant, counting from the first. The first set of two plants was immediately processed for brush-sampling by cutting off all live (any remaining green) leaves on one side of each plant (= two half-plants). These leaves were cut into 15- to 20-cm sections, carefully stacked, sealed in 4-l plastic bags, and transported to the laboratory in an ice chest. The second set of two plants was visually examined for arthropods associated with spider mite colonies. Arthropods were counted and samples were collected in 70% methanol using a hand-held aspirator for species determination. Arthropods were keyed to family and submitted to the BBII, USDA for further identification (Table 3).

Laboratory procedures. Field-processed leaf samples were brushed immediately upon returning to the laboratory. Leaf samples that could not be brushed immediately were refrigerated at 4 to 6°C. All samples were brushed within 17- to 24-hours following field collection. Glass collection disks had been washed in a Sparkleen® solution, dried, and coated with a fine mist of a 1:10 (v:v) mixture of Armul® 535 and 99% ethanol (caution: flammable). Previously refrigerated leaf samples were air-dried until no free moisture remained. Individual leaf sections were lowered into the machine, pressed against the single brush

with the thumb, and slowly withdrawn, allowing several revolutions of the collection disk for even distribution of dislodged mites. While the machine continued to run, and before removal of the collection disk, the side-shields and the brush were cleaned with a test tube brush to ensure mite transfer and to avoid cross-contamination.

Collection disks were removed from the brushing machine and centered over a 200-section equal area disk (Morgan et al., 1955). Spider mites (immature or adults), predator mites, and their eggs were counted using 1/40 (high mite numbers), 1/20 (medium mite numbers), or 1/10 (low mite numbers) of the disk area. Mite and egg counts were converted to mites per plant for comparison between samples. Subsamples of 25 to 50 live and 25 to 50 dead mites were mounted in Hoyer's mounting medium, and ringed with Permount®. Mounts were evaluated microscopically (Olympus® BH/BHA with phase contrast) to determine mite species, development stage (immature/male/mature female), and the presence of Neozygites sp. A mite was considered to be infected if it contained hyphal bodies, resting spores, or had capilliconidia attached to its integument.

RESULTS AND DISCUSSION

1984 Results.

Samples. Refrigeration of samples for two weeks to induce resting spores was unnecessary because resting spores formed naturally during an epizootic. It appears that freezing may have burst young hyphal bodies, making their detection difficult. In addition, a saprophytic fungus (Alternaria spp.) developed rapidly on the refrigerated leaf samples and interfered with disease detection. Other aspects of the sampling procedure were cumbersome so new field and laboratory procedures were developed for 1985.

Area weather (Norwood 1985, Hadeen 1985). The fall of 1983 was very dry and the winter of 1983-84 was unusually cold. The months of July through September were slightly cooler than normal, but precipitation was considerably below normal. No significant rainfall was recorded at GCX between 28 July and 5 October, and some of the highest temperatures of the summer occurred in late August.

BGM and TSM populations. Due to the dry fall (Buschman and Dick in press) and the unusually cold winter of 1983-84, very low numbers of spider mites overwintered. Initially, spider mite populations were slow to develop, but by mid-season numbers of adult female mites reached 50 to 1000 per plant (moderate infestation), and by the end of the season, numbers of adult female mites reached 1400 to 3000 per plant (heavy infestation). BGM and TSM were present at all study sites in 1984, but numbers of TSM were very low (0.15 to 37 adult females per plant) at STA and STB. Based on the numbers of mites present when epizootics were

detected in 1985 (Figs. 25 to 31), the number of mites per plant at most study sites in 1984 would appear to have been able to support an epizootic had conditions been otherwise favorable.

The effect of weather on Neozygites sp. epizootics. Neozygites sp. was not detected in regular weekly samples in 1984, but a very low level was detected in a related laboratory study using material collected 8 September at FIA (SE). The absence of fungal epizootics in 1984 was probably due to insufficient periods of humidity when epizootics have historically been observed in the western Great Plains. According to weather data for the period, there were very few times ambient relative humidity exceeded 80% (=90% canopy relative humidity) for more than 8 hours between 15 August and 8 September, which was definitely below the levels postulated other researchers to favor epizootics (Kenneth et al. 1972, Wilding 1981, and Smitley 1986a). Based on a liberal interpretation of humidity thresholds postulated by Missioner et al. (1970) and Brandenburg & Kennedy (1982), the humidity levels between 15 August and 8 September 1984 (Fig. 32) would not be expected to support Neozygites sp. epizootics. The effect of temperature was probably limited to its influence on atmospheric humidity since the fungus is capable of sporulation and infection at temperatures encountered during this period (Smitley 1986a).

1985 Results.

Samples. Use of the modified leaf brushing machine in 1985 produced favorable results. The machine facilitated counting of the high numbers of mites encountered (up to 33,000 per plant) at some study

sites in 1985 and enabled relatively easy estimation of adult female mites, immature mites, and mite eggs. Estimates of mite numbers using the brushing machine compared favorably to visual counts of adult female mites (unpublished data).

Area weather (Norwood 1986, Hadeen 1986). Near-record rainfall occurred in October 1984 following the dry summer, which probably contributed to the establishment of mite overwintering habitat. Winter temperatures of 1984-85 were much warmer than those of 1983-84, which probably contributed to increased survival of overwintering mites. Temperatures during the study period (July to early-September) were well below normal and August and September 1985 rainfall was above normal. Heavy rain accompanied by high wind apparently occurred at STA (NW) between 19 and 26 July. The corn plants showed evidence of leaf-shredding due to high wind, and spider mite numbers declined sharply (Fig. 28).

BGM and TSM populations. Numbers of overwintering BGM and TSM in non-crop hosts were six times greater in 1984-85 than in 1983-84 (Buschman and Dick in press). BGM were present at all seven study sites in 1985 (Figs. 25 to 31), and populations followed two general trends. In the first trend (Type A), populations increased slowly early in the season, accelerated in late-June or early-July, and reached a peak between late-July and early-September (Figs. 25, 26, and 31). In the second trend (Type B), BGM numbers increased rapidly in mid- to late-June but then decreased rapidly in mid-season (Figs. 27 to 29, 30). BGM numbers declined to zero in mid-season at three of the four Type B sites (Figs. 28 to 30). Except for STA (NW) at which mite numbers remained at zero, numbers of BGM began to increase again in late August.

TSM were present at five of the seven study sites in 1985 (Figs. 25, 26, and 29 to 31), and populations followed a single general trend. TSM populations generally began to develop later than BGM, rapidly increased to a peak in early- to mid-August, then rapidly decreased in mid-August to early-September. At HAA (NE and SW), TSM numbers reached a peak when BGM numbers were at their lowest (Figs. 29 and 30).

Because epizootics occured in Type A and B BGM populations and in populations of TSM, it appears that population development pattern is not a significant factor in the initiation of epizootics. It does appear that epizootics generally coincided with peak mite numbers (Figs. 25 to 27, 29 to 31). The concept of density dependence in relation to propagation of epizootics has received much attention, but whether or not it occurs is debated (Wilding 1981). Brandenburg & Kennedy (1982) and Smitley (1986b) hint at density dependence. The results of this study do not clearly support or reject density dependence as a factor in epizootic development.

Predators and associated arthropods. Representatives of at least 17 species or species complexes were found (Table 3). Five species or species complexes were more frequently collected (Figs. 25 to 31). These are, in decreasing order of abundance: A phytoseiid complex (mainly Neoseiulus fallacis Garman); a complex of two predatory and six phytophagous thrips; Orius insidiosis (Say); an undetermined psocid; and a complex of Stethorus spp., including Stethorus punctum punctum (LeConte). Of these five species complexes or species, four were known mite predators.

Neoseiulus fallacis is one of the most common native predators of BGM (and possibly of TSM) in the western Great Plains. It was the most abundant predatory mite in this study and was the second most abundant predatory mite observed by Pickett (1985). W. R. Enns, in a communication to K. O. Bell concerning predatory mites in southwest Kansas (Stevens Co.), stated that Neoseiulus fallacis is the most abundant predatory mite where pesticides are used. Pickett (1985) cites a study that reports native phytoseiids did not contribute significantly to mite suppression in Texas. Phytoseiids appear to respond numerically in this study (Figs. 25 to 31) indicating that the phytoseiid complex was reacting to increasing spider mite density. The phytoseiid population apparently follows BGM numbers (Figs. 25, 27, and 28), TSM numbers (Figs. 26, 29, and 30), or both (Fig. 31). In this study, the phytoseiid complex may have had a significant negative impact on numbers of mites at all but FIA (SE) and HAA (SW) (Figs. 25 and 30).

Eight species of thrips were present, including Scolothrips pallidus and Scolothrips spp. (both predatory) and Frankliniella occidentalis (a facultative predator). Frankliniella occidentalis is an important predator of spider mites on cotton, consuming 40 mite eggs per day per predator (Trichilo & Leigh 1986). It appears that the thrip complex may have had a significant early-season impact on mite populations (Figs. 25 to 28). This portion of the study should be repeated using a sampling technique capable of accounting for relative numbers of each thrip species.

The remaining three species or species complexes were present at relatively low levels compared to Neoseiulus fallacis and thrips (Figs.

25 to 31). Though O. insidiosis is a predator of small insect and mite eggs it did not appear to affect spider mites in 1985. Numbers at most study sites remained low and were relatively constant through much of the study period. Significant numbers of S. punctum punctum were present only at HAA (NE), and a slight numerical response to spider mite density occurred (Fig. 29). A few Orius were occasionally collected at other sites but had no apparent effect on populations of spider mites.

A psocid, the only non-predator regularly collected during arthropod sampling, was present at three of six study sites that developed Neozygites epizootics (Figs. 25, 26, and 29). It was absent from the remaining study sites (Figs. 27, 28, 30, and 31). Psocids feed on fungi, detritus, cereals, and pollen. They probably have no direct effect on spider mite numbers, but there may be an indirect effect involving Neozygites. There appears to be a close temporal relationship between the occurrence of the psocid and the period during which epizootics occur (Figs. 25, 26, and 29). It would be informative to investigate the relationship of this particular psocid to Neozygites spp.

Effect of insecticides and miticides. Insecticide and miticide applications for 1985 are summarized in Table 2. Six study sites received two pesticide applications - the seventh received only a single late-season miticide application. In all cases of two applications, the first was a miticide and the second a corn borer insecticide, miticide, or a combination of the two. It was difficult to separate the impact of pesticides from the impact of predators and Neozygites sp. Mite populations at six of the seven study sites appeared to be affected only

slightly, if at all, by insecticide or miticide applications (Figs. 25, 26, and 28 to 31). Some of the early- to mid-season declines in numbers of mites appeared to coincide with increasing numbers of predators as much as with pesticide applications (Figs. 25 to 28). One of the late-season declines in numbers of mites appeared to coincide with increasing numbers of predators and the occurrence of Neozygites epizootics as much as with pesticide applications (Figs. 31). Two of the mid- to late-season declines in numbers of mites appeared to coincide only with increasing numbers of predators and the occurrence of Neozygites (Figs. 30 and 31).

The effect of pesticides on numbers of predators was much more apparent. At four of five sites the numbers of thrips and/or phytoseiids appeared to have been substantially reduced by pesticide applications (Figs. 25 to 28, and 31). At HAA (NE and SW), a severe early-season mite infestation necessitated early spraying. Since both spray dates were prior to the time weekly sampling began, data for early-season spray effects on predators is unavailable.

The effect of Neozygites sp. on BGM and TSM. Neozygites sp. epizootics developed in BGM at five of the seven study sites in 1985 (Figs. 25 to 27, and 30 to 31). At study sites where epizootics (regularly detectable levels) occurred in populations of BGM, the infection rate was 0 to 50% (live mites) and 0 to 81% (dead mites). Of five study sites at which TSM occurred in 1985, Neozygites sp. epizootics developed at four. The infection rate in TSM was 0 to 13% (live mites) and 0 to 33% (dead mites). These infection rates are

similar to those observed by Brandenburg & Kennedy (1982) for N. floridana in TSM (0 to 71%).

No enzootic levels (undetectable or occasionally detectable) of Neozygites were found in 1984 or 1985, nor were enzootic levels detected during three years of studying overwintering habitat in southwest Kansas (Buschman & Dick, unpublished data). Only two researchers report enzootic levels during the winter. Weiser (1968) and Brandenburg & Kennedy (1981) report overwintering as hyphal body-filled cadavers or resting spores and as hyphal body-filled cadavers near live mites, respectively. Smitley (1986b) reports some level of infection throughout the summer when moisture is adequate, but cites Brandenburg & Kennedy (1981) for the overwintering mechanism. Investigation of an overwintering mechanism or a detailed search for an enzootic level in the western Great Plains should receive priority.

A low level of Neozygites sp. was first detected in BGM at Finney A (SE) on 14 August 1985 during a related study. Its first detection during regular weekly sampling was 15 August (Fig 29). The fungus was subsequently detected at various sites and dates throughout the remainder of the sampling period (Figs. 25 to 27 and 29 to 31). In 1985, the dates of first Neozygites sp. detection ranged from 14 to 31 August. If a 10%-level of infection is estimated and used to signal the onset of an epizootic, it appears that epizootics began from 15 to 27 August. This is a considerably shorter interval than the 50-day period of epizootic onset (20% infection level) reported by Smitley (1986b).

Effect of weather on Neozygites sp. epizootics. Periods of high relative humidity are thought to be the most important factor in the

initiation and propagation of Entomophthoraceous epizootics (Wilding 1981). In this study the onset of epizootics generally coincided with a period of $\geq$10 hours per day of ambient relative humidity $\geq$80% ($\geq$90% canopy humidity) between 14 and 27 August (Figs. 25-32). Three significant rains occurred during this 14-day period. The general weather conditions postulated as necessary for an epizootic to occur (Missioner et al. 1970, Brandenburg & Kennedy 1982, and Smitley 1986a), apparently were met. Though epizootics occurred, there appeared to be factors other than humidity involved. Two similar periods of humidity (and rainfall) on 14 to 24 July, and on 28 July to 7 August that appeared to meet the general weather criteria for an epizootic to occur (Missioner et al. 1970, Brandenburg & Kennedy 1982, and Smitley 1986b) did not result in detectable disease, even though apparently adequate mite numbers (Figs. 25 to 27 and 29 to 31) were present. Why epizootics did not occur earlier is unknown. Also, some of the highest rates of infection were observed during a dry period near the end of the season (Figs. 25, 26, 30, and 31). A possible explanation for continuation of epizootics during dry periods is that once capilliconidia have been formed, they may continue to remain viable for some time (Uziel & Kenneth 1986). These viable capilliconidia may then be able to infect mites due to the boundary layer of moisture adjacent to the corn leaf surface (Wagoner 1965). Future laboratory and field studies will be needed to further define the parameters of successful epizootics.

Effect of Neozygites sp. on BGM and TSM populations. Because of interactions between weather, host plant, predators, and pesticides in 1984 and 1985, it was difficult to separate the effect of Neozygites sp.

from the effect of other factors. At FIA (SW) and FIA (SE), infection occurred in a relatively high percentage of mites, yet numbers of live mites continued to increase throughout the epizootic at FIA (SW) (Figs. 25 and 26). This suggests that even though environmental conditions favored relatively high rates of infection, the epizootic was not capable of limiting the mite population under those conditions. Only at a FIA (SE) did the fungus appear to have a significant impact on numbers of mites (Fig. 25). FIA (SE) is the only study site at which predatory arthropods did not appear to be associated with a decline in mite numbers. It is also the only site at which rate of infection appeared to be sufficient to reduce numbers of mites. Except at FIA (SE), decline in numbers of mites appeared to be due to combinations of interactions between predatory arthropods, climatic factors, pestcides, and other factors. One factor that should not be overlooked is the effect of host plant senescence. As host senescence occurs, mites begin to migrate from corn plants, leading to rapid decrease in numbers of mites per plant. Brandenburg and Kennedy (1982) report that the effect of host senescence is inseparable from the effect of Neozygites sp. on mite populations, but Smitley (1985) presents evidence that Neozygites sp. causes mite population reduction. Additional studies are needed to separate the effects of host senescence from the effects of Neozygites sp. on spider mite populations.

CONCLUSIONS AND ADDITIONAL STUDIES

Observations made during this study established a broad overview of Neozygites epizootics in the western High Plains. There are

several general conclusions that may be made regarding the fungus, its host, and the environment. First, epizootics do not occur every year, but when they do occur, they appear to coincide with periods of high relative humidity in mid-August to early September. Second, epizootics generally occur after much of the mite damage to corn has already occurred. Third, dramatic declines in numbers of mites due solely to Neozygites sp. may not be common. Fourth, there appear to be several unknown variables, including the effects of abiotic factors, the fungal reservoir in the absence of enzootic levels (alternate hosts?), and the role of the host plant itself.

Other studies should be conducted in order to attempt to explain the absence of enzootic levels and the absence of epizootics earlier in the season. Though epizootics usually occur too late to prevent corn damage, the effect on the numbers of mites that overwinter should be investigated. The possibility of alternate fungal hosts should be examined. This subject has received little attention apparently because Entomophthoraceous fungi are thought to be relatively family-or order-specific (Wilding 1981). Fungi within the genus Neozygites are known to infect several arthropods orders in a variety of climates. A study cited by Tsintsadze & Vartapetov (1976) demonstrated cross infectivity of Entomophthora thaxteriana Petch between plant lice (aphididae?) and Tetranychus urticae and Tetranychus cinnabarinus Boisd.. In southwest Kansas, near the first week of August 1985, Neozygites-infected aphids were collected from corn plants at FIA (SE). In 1986, Neozygites-infected aphids were again observed on corn plants at FIA (SE) shortly after corn tasseling. At the same time, Neozygites sp. was observed

infecting several thrips feeding on Johnsongrass adjacent to the FIA (SE) study site. The relevance of these observations to the occurrence of spider mite epizootics is unknown, but they are worthy of future investigation.

Though the situation appears complicated, it seems that a computer model alerting mite workers to (but not predicting) conditions favorable for the onset of epizootics could be developed. Such a model could be used by entomologists and agricultural consultants in the western Great Plains when interpreting the results of experiments or when making late-season miticide recommendations.

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Table 2. Insecticides and miticides applied to 1985 study sites.

Study Site	Treatment Date	Insecticide/ Miticide	Application Rate
Finney County A (SE, SW) (FIA)	23 Jul 1985	Propargite	0.76 kg [AI]/ha
	14 Aug 1985	Carbofuran	0.34 kg [AI]/ha
Stanton County A (SW, NW) (STA)	13 Jul 1985	Propargite	0.76 kg [AI]/ha
	4 Aug 1985	(Carbofuran + Methidathion)	(0.34 kg [AI]/ha + 0.23 - 0.45 kg [AI]/ha)
Haskell County A (NE, SW) (HAA) (SW)	24 Jun 1985	Propargite	0.76 kg [AI]/ha
	11 Jul 1985	Methidathion	0.45 kg [AI]/ha
(NE)	11 Jul 1985	Methidathion	0.91 kg [AI]/ha
Finney County B (NW) (FIB)	at planting	Fonofos	1.40 kg [AI]/ha
	15 Aug 1985	Dimethoate	0.23 kg [AI]/ha
Ford County A (NE, SW) (FOA)	12 Jul 1985	Propargite	0.38 - 0.76 kg [AI]/ha
Finney County C (NE, SW) (FIC)	16 Jul 1985	Propargite	0.38 - 0.76 kg [AI]/ha

Table 3. Predatoroy and incidental arthropods associated with spider mite colonies on southwest Kansas field corn.

IDENTIFICATION	HOST	IDENTIFIED BY:**
Acari:		
Phytoseiidae		
Neoseiulus fallacis Garman	Acari	H. A. Denmark (a)
Araneae:		
Araneidae		
Undetermined	Generalist predator	J. Coddington (b)
Coleoptera:		
Coccinellidae		
Hippodamia parenthesis Guerin	Generalist predator	R. D. Gordon (c)
Scymnus sp. larvae	Aphids	D. M. Anderson (c)
Stethorus punctum punctum (LeConte)	Acari, Thysanoptera	R. D. Gordon (c)
Corylophydae		
Molamba sp.*	Fungi*	R. D. Gordon (c)
Phalacridae		
Phalacrus sp.	Compositae Flowers	W. E. Steiner (b)
Hemiptera:		
Anthocoridae		
Orius insidiosus Say	Insect and mite eggs	T. J. Henry (c)

*Irrelevant to this study.
**Identifier affiliation:

(a). Florida Department of Agriculture and Consumer Services, Gainesville. Cooperating Acarologist for the Systematic Entomology Laboratory, BBII, USDA.
(b). Smithsonian Institution
(c). Research Entomologist, Systematic Entomology Laboratory, BBII, USDA

Table 3. Predatoroy and incidental arthropods associated with spider mite colonies on southwest Kansas field corn, continued.

IDENTIFICATION	HOST	IDENTIFIED BY **
Hymenoptera:		
Aphidiidae		
Undetermined*	Aphid Parasites*	P. M. Marsh (c)
Formicidae		
Hypoponera sp. workers	Workers carnivorous	D. R. Smith (c)
Mymaridae		
Polynema sp*	Insect Egg Parasites*	M. E. Schauff (c)
Neuroptera:		
Hemerobiidae		
Undetermined	Generalist predator	O. S. Flint (b)
Chrysopidae		
Chrysopa carnea Steph.	Generalist predator	O. S. Flint (b)
Thysanoptera		
Thripidae		
Anaphothrips sp.	Phytophagous	S. Nakahara (c)
Undetermined	No information	S. Nakahara (c)
Frankliniella exigua Hood	Phytophagous	S. Nakahara (c)
Frankliniella occidentalis (Pergande)	Facultative predator (Trichilo & Leigh 1986)	S. Nakahara (c)
Frankliniella tenuicornis male	Phytophagous	S. Nakahara (c)
Proscirtothrips zeae (Moulton)	Phytophagous	S. Nakahara (c)
Scolothrips pallidus (Beach)	Acari	S. Nakahara (c)
Scolothrips sp.	Acari	S. Nakahara (c)
Aeolothripidae	Phytophagous	S. Nakahara (c)
Undetermined	Generalist predator	S. Nakahara (c)

*Irrelevant to this study.
**Identifier affiliation.

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(b). Smithsonian Institution
(c). Research Entomologist, Systematic Entomology Laboratory, BBII, USDA

Figure 25. Finney County A (SE). Comparison of humidity and precipitation, populations of live and dead BGM and TSM, percent of live and dead BGM and TSM infected, and predatory and associated arthropod populations by date. Arrows indicate insecticide/miticide application date: 23 July treatment 0.76 kg [AI]/ha propargite; 14 August treatment 0.34 kg [AI]/ha carbofuran.

HRS ABOVE 80% RH
GCX PPT.
LAKIN PPT.
GCX HOURS ABOVE 80% RH
PRECIPITATION (mm)
LIVE
% LIVE INFECTED
DEAD
% DEAD INFECTED
BGM/PLANT x 1000
% INFECTED x 10
TSM/PLANT x 10
PREDATOR MITE COMPLEX
THRIPS COMPLEX
Orius insidiosis
COLEOPTERA
PREDATORS/PLANT x 10
6/30
7/10
7/20
7/30
8/9
8/19
8/29
9/8
MONTH/DAY

Figure 26. Finney County A (SW). Comparison of humidity and precipitation, populations of live and dead BGM and TSM, percent of live and dead BGM and TSM infected, and predatory and associated arthropod populations by date. Arrows indicate insecticide/miticide application date: 23 July treatment 0.76 kg [AI]/ha propargite; 14 August treatment 0.34 kg [AI]/ha carbofuran.

GCX HOURS ABOVE 80% RH
HRS. ABOVE 80% RH
GCX PPT.
LAKIN PPT.
PRECIPITATION (mm)
BGM/PLANT x 1000
LIVE
% LIVE INFECTED
DEAD
% DEAD INFECTED
% INFECTED x 10
TSM/PLANT x 10
LIVE
% LIVE INFECTED
DEAD
% DEAD INFECTED
% INFECTED x 10
PREDATORS/PLANT x 10
PREDATOR MITE COMPLEX
THRIPS COMPLEX
Orius insidiosis
PSOCOPTERA
6/30 7/10 7/20 7/30 8/9 8/19 8/29 9/8
MONTH/DAY

Figure 27. Stanton County A (SW). Comparison of humidity and precipitation, populations of live and dead BGM, percent of live and dead BGM infected, and predatory and associated arthropod populations by date. Arrows indicate insecticide/miticide application date: 13 July treatment 0.76 kg [AI]/ha propargite; 4 August treatment 0.34 kg [AI]/ha carbofuran plus 0.23 to 0.45 kg [AI]/ha methidathion.

HRS. ABOVE 80% RH
LAKIN PPT.
BIG BOW PPT.
CCX HOURS ABOVE 80% RH
PRECIPITATION (mm)
LIVE
% LIVE INFECTED
DEAD
% DEAD INFECTED
BSM/PLANT x 100
% INFECTED x 10
PREDATOR MITE COMPLEX
THRIPS COMPLEX
Orius insidiosis
PSOCOPTERA
PREDATORS/PLANT x 10
8/30
7/10
7/20
7/30
8/9
8/19
8/29
9/8
MONTH/DAY

Figure 28. Stanton County A (NW). Comparison of humidity and precipitation, populations of live and dead BGM, percent of live and dead BGM infected, and predatory and associated arthropod populations by date. Arrows indicate insecticide/miticide application date: 13 July treatment 0.76 kg [AI]/ha propargite; 4 August treatment 0.34 kg [AI]/ha carbofuran plus 0.23 to 0.45 kg [AI]/ha methidathion.

HRS. ABOVE 80% RH
LAKIN PPT
BIG BOW PPT
GCK HOURS ABOVE 80% RH
PRECIPITATION (mm)
LIVE
% LIVE INFECTED
DEAD
% DEAD INFECTED
BGM/PLANT x 10
% INFECTED x 10
PREDATOR MITE COMPLEX
THRIPS COMPLEX
Orius insidiosis
PSOCOPTERA
PREDATORS/PLANT x 10
6/30
7/10
7/20
7/30
8/9
8/19
8/29
9/8
MONTH/DAY

Figure 29. Haskell County A (NE). Comparison of humidity and precipitation, populations of live and dead BGM and TSM, percent of live and dead BGM and TSM infected, and predatory and associated arthropod populations by date. Arrows indicate insecticide/miticide application date: 24 June treatment 0.76 kg [AI]/ha propargite; 11 July treatment 0.91 kg [AI]/ha methidathion.

GCX HOURS ABOVE 80% RH
PRECIPITATION (mm)
HRS ABOVE 80% RH
GCX PPT.
SUBLETTE PPT.
BSM/PLANT x 100
% INFECTED x 10
LIVE
% LIVE INFECTED
DEAD
% DEAD INFECTED
TSM/PLANT x 1000
% INFECTED x 10
LIVE
% LIVE INFECTED
DEAD
% DEAD INFECTED
PREDATORS/PLANT x 10
PREDATOR MITE COMPLEX
THRIPS COMPLEX
Orius insidiosis
PSOCOPTERA
Stethorus sp.
6/30
7/10
7/20
7/30
8/9
8/19
8/29
9/8
MONTH/DAY

Figure 30. Haskell County A (SW). Comparison of humidity and precipitation, populations of live and dead BGM and TSM, percent of live and dead BGM and TSM infected, and predatory and associated arthropod populations by date. Arrows indicate insecticide/miticide application date: 24 June treatment 0.76 kg [AI]/ha propargite; 11 July treatment 0.45 kg [AI]/ha methidathion.

HRS. ABOVE 80% RH
90% PPT.
SUBLETTE PPT.
60% HOURS ABOVE 80% RH
PRECIPITATION (mm)
LIVE
% LIVE INFECTED
DEAD
% DEAD INFECTED
BSM/PLANT x 100
% INFECTED x 10
LIVE
% LIVE INFECTED
DEAD
% DEAD INFECTED
TSM/PLANT x 1000
% INFECTED x 10
PREDATOR MITE COMPLEX
THRIPS COMPLEX
Orius insidiosis
PSOCOPTERA
PREDATORS/PLANT x 10
6/30
7/10
7/20
7/30
8/9
8/19
8/29
9/8
MONTH/DAY

Figure 31. Finney County B (NW). Comparison of humidity and precipitation, populations of live and dead BGM and TSM, percent of live and dead BGM and TSM infected, and predatory and associated arthropod populations by date. Arrow indicates insecticide/miticide application date: 15 August treatment 0.23 kg [AI]/ha dimethoate.

HRS ABOVE 80% RH
GCX PPT
LAKIN PPT
GCX HOURS ABOVE 80% RH
PRECIPITATION (mm)
LIVE
% LIVE INFECTED
DEAD
% DEAD INFECTED
BSM/PLANT x 1000
% INFECTED x 10
TSM/PLANT x 1000
% INFECTED x 10
PREDATOR MITE COMPLEX
THRIPS COMPLEX
Orius insidiosis
PREDATORS/PLANT x 10
6/30
7/10
7/20
7/30
8/9
8/19
8/29
9/8
MONTH/DAY

Figure 32. Three year comparison of Garden City Experiment Station hours per day of ambient relative humidity $\geq$ 80% (= $\geq$90% canopy relative humidity) and precipitation at the Garden City Experiment Station and Lakin, KS. Horizontal lines at 8 and 10 hours per day of ambient relative humidity $\geq$ 80% bracket the humidity levels postulated by Missioner et al. (1970) and Brandenburg and Kennedy (1982) to favor development of entomophthoraceous and Neozygites epizootics.

Legend: Humidity
GCX Precipitation
Lakin Precipitation
1984
1985
1986
GCX HOURS/DAY ABOVE 80% RH
PRECIPITATION (mm)
6/30
7/10
7/20
7/30
8/9
8/19
8/29
9/8
MONTH/DAY

CHAPTER 4

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

The objectives of this study were to identify and describe the fungal pathogen infecting BGM, and to examine the field ecology of this fungus in the western Great Plains. These objectives were fulfilled to the degree that could be expected of a study this broad in scope.

The fungal pathogen most closely resembled Neozygites adjarica, originally described in the southern USSR by Tsintsadze & Vartapetov (1976). It also closely resembled a fungus found by Keller & Wuest (1983) to closely resemble Neozygites adjarica. The fungus was typical of the family Entomophthoraceae and probably belongs to the new family, Neozygitaceae, proposed by Ben-Ze'ev (1986). It is definitely a member of the genus Neozygites. The general biology of the Kansas/Texas fungus within BGM was very similar to that reported by other researchers for Neozygites spp. in TSM, Oligonychus hondoensis, Mononychelus progressivus, and Tetranychus evansi. Morphological characteristics that set this fungus apart from N. floridana and N. tetranychi were the common occurrence of resting spores and rhizoids, smooth-walled spericaltoovoid resting spores, and smooth-walled primary conidia and capilliconidia. Taxonomic changes are to be expected as more is learned about the Neozygites spp. that infect plant feeding mites.

In studying the field ecology of the fungus in relation to BGM and TSM, several factors were briefly investigated, and all appeared to play a role in the complicated population dynamics of corn spider mites. Factors investigated included precipitation and humidity, mite populations, the incidence of fungal disease, arthropods associated with

mite colonies, and the effects of insecticides and miticides. In this study predatory arthropods (and in one case abiotic factors) appeared to have the greatest negative impact on spider mite populations, in contrast to prior field observations that suggest this Neozygites sp. commonly decimates spider mite populations.

There are several general conclusions that can be made in regard to the Neozygites sp. observed in this study. First, Neozygites sp. is capable of causing high spider mite mortality under certain conditions. Second, sporadic rainfall and subsequent low humidity prevent epizootics from occurring regularly in the western Great Plains. Third, without some ability to manage humidity, it is unlikely that fungal epizootics will result in timely mite control (prior to crop damage). Fourth, the dramatic epizootics observed from time to time may be due to coincidence of several factors rather than to any individual factor. Finally, there is much to be learned in regard to how factors in the agroecosystem influence each part of the mite/fungal pathogen interaction.

Based on the results of this investigation, it would be easy to conclude that Neozygites sp. epizootics are of little practical value if mite control in the traditional sense is the only goal. It is evident, however, that the fungal epizootics fit into a larger pattern of natural mite suppression resulting from a number of environmental factors. Therefore, it is important that entomologists and others involved with agricultural pest control and research be aware of the fungus and the general conditions favorable for development of epizootics, and to use this awareness in management of late-season spider mite infestations. It is important that mite researchers be aware of the potential impact

of the fungus on field plot research findings.

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IDENTIFICATION AND FIELD ECOLOGY OF A FUNGAL PATHOGEN OF *OLIGONYCHUS PRATENSIS* AND *TETRANYCHUS URTICAE* (ACARI: TETRANYCHIDAE): *NEOZYGITES* SP. (ENTOMOPHTHORALES: NEOZYGITACEAE):

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ABSTRACT

Major fungal epizootics periodically occur in populations of tetranychid mites in the western Great Plains of North America. Samples of the fungal pathogen were collected in Kansas and Texas. Short and long hyphal bodies, primary conidia, capillary conidiophores, capilliconidia, resting spores, and rhizoids typical of the genus Neozygites Witlaczil were described. Based on the surface texture of capilliconidia and resting spores, the shape of resting spores, and the occurrence of rhizoids, this fungus more closely resembles Neozygites adjarica than N. tetranychi or N. floridana. Because conidial dimensions overlap those fungi reported as N. floridana and N. tetranychi, and because the degree of morphological plasticity of members of this genus is not known, a more positive identification is not possible at this time.

Corn fields were monitored for the effects of a fungal spider mite pathogen, Neozygites sp., on populations of Oligonychus pratensis and Tetranychus urticae. The related factors of relative humidity and precipitation, predatory and incidental arthropods, and the effects of insecticides and miticides were also monitored with respect to their overall effect on the mite-pathogen relationship. There is a complex combination of interactions among these factors. Several fungal epizootics were observed, apparently coinciding with mid-August or later periods of 8 to 10 hours per day of $\geq$ 80% ambient relative humidity. No fungal epizootics were observed earlier than mid-August even when these periods of relative humidity occurred. At only one study site did it

appear that the fungus was the major mortality agent in 1984 and 1985. This is in contrast to prior field observations which indicated that Neozygites sp. was capable of decimating spider mite populations. In this study, combinations of factors other than Neozygites sp., including predatory arthropods, weather, and possibly host-plant senescence, appeared to be the most important agents of mortality. Insecticide and miticide applications appeared to have a much greater impact on predatory and incidental arthropods than on spider mites. Though Neozygites sp. appeared to have no regularly occurring negative impact on numbers of spider mites, it appeared to be one of a combination of factors that, as a whole, are capable of suppressing spider mite populations. Those who monitor spider mites or conduct spider mite research should be aware of the potential impact of Neozygites sp. on spider mite populations.